vacuum. At a small volume a colorless product crystallized out. This product was filtered off, washed with water and recrystallized from acetic acid. The melting point was 204° with sublimation and decomposition; 150 mg. was obtained.

Anal. Calcd. for $C_9H_7NO_2$: N, 8.70; neut. equiv., 161. Found: N, 8.67; neut. equiv., 160, 165.

The **methyl ester** was prepared by refluxing for one hour with methanol and a trace of sulfuric acid. It crystallized from aqueous acetone as platelets; m. p. 151°.

Anal. Calcd. for $C_{10}H_9NO_2$: N, 8.00. Found: N, 8.02.

The **ethyl ester** was prepared in a similar way with ethanol and sulfuric acid. Needles were obtained on recrystallization from aqueous ethanol or benzene–petroleum ether; m. p. 121°.

Anal. Calcd. for $C_{11}H_{11}NO_2$: N, 7.40. Found: N, 7.36.

These compounds were proved to be identical with indole-2-carboxylic acid, m. p. 204°, indole-2-carboxylic acid methyl ester, m. p. 151°, and indole-2-carboxylic acid ethyl ester, m. p. 122°, by mixed melting point determinations with synthetic samples.

Synthesis of the Hydriodic Acid Reduction Product of Gliotoxin.—After the isolation of indole-2-carboxylic acid from the strong aqueous alkaline hydrolysis of the 13-carbon acid it was found impossible to obtain any further crystalline material. In view of the possibility that the remainder of the molecule might be either α- or β-N-methylalanine the following synthesis was carried out. Indole-2-carboxylic acid was synthesized by the method of Reissert[6] and converted to the acid chloride by the action of thionyl chloride. To 25 ml. of dry ether containing 1.0 g. of thionyl chloride, 175 mg. of finely powdered indole-2-carboxylic acid was slowly added. Solution occurred readily and after all the acid had been added the mixture was allowed to stand at room temperature for forty minutes before the ether and excess thionyl chloride were removed in vacuum. The slightly pigmented semi-crystalline residue was redissolved in dry ether and used without further purification since it decomposed readily. Since the 13-carbon acid obtained from gliotoxin possessed no optical activity, inactive α-N-methyl alanine ethyl ester, prepared by the method of Zelinsky, *et al.*,[7] was used. An ethereal solution of 175 mg. of this ester was added to the solution of indole-2-carboxylic acid chloride. The mixture warmed slightly and became cloudy. It was allowed to stand at room temperature for one hour after which the ether and excess amino acid ester were removed in vacuum. The crystalline residue was taken up in absolute alcohol and recrystallized by the addition of water to the warm solution. Colorless needles melting at 126° were obtained. The yield was 148 mg. and represented 55% of the theoretical yield on the basis of the indole-2-carboxylic acid used.

This synthetic ester agreed exactly in all properties with the ester of the 13-carbon acid and showed no depression in a mixed melting point determination. On saponification it yielded the identical 13-carbon acid; m. p. 187°.

It was observed in a second preparation of this ester that if the alcoholic solution of the initial condensation product of the indole-2-carboxylic acid chloride and *dl*-α-N-methyl alanine ester was allowed to stand for thirty-five hours, instead of obtaining the open chain ester of the 13-carbon acid, a product melting at 122° and identical with the hydriodic acid reduction product of gliotoxin was obtained. The presence of a small amount of hydrochloric acid was apparently responsible for this cyclization since the addition of solid sodium carbonate to the ethereal solution, after condensation, to remove all traces of hydrochloric acid prevented the cyclization. The neutral, cyclized product was then obtained from the ester by allowing an alcoholic solution containing 1% hydrochloric acid to stand for forty-eight hours. From 750 mg. of indole-2-carboxylic acid, 525 mg. of the cyclized product was obtained; this represents 50% of the theoretical yield.

(6) A. Reissert, *Ber.*, **30**, 1036 (1897).

(7) Zelinsky, Annenkoff and Kulikoff, *Z. physiol. Chem.*, **73**, 468 (1911).

Summary

Treatment of gliotoxin with phosphorus and hydriodic acid in acetic acid gave a crystalline product in which analysis showed that no sulfur and two less oxygen atoms were present; but the same number of carbons as in gliotoxin were found. This product was degraded stepwise to N-(indole-2-carboxoyl)-N-methyl-α-alanine and to indole-2-carboxylic acid. The crystalline reduction product, 2,3-dimethyl-1,4-diketotetrahydropyrazino-[1,2-a]-indole (I) and the several degradation products have been identified by synthesis.

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

Gliotoxin, the Antibiotic Principle of *Gliocladium fimbriatum*. IV. The Structure of Gliotoxin: The Action of Selenium

BY JAMES D. DUTCHER,[1] JOHN R. JOHNSON AND WILLIAM F. BRUCE

In seeking to establish the structure of gliotoxin, the antibiotic substance produced by the fungus *Gliocladium fimbriatum*,[2] a distillation with selenium was carried out. A curious but informative reaction occurred which yielded a product that shed considerable light upon the structure of the gliotoxin molecule and served to verify the observations previously reported for the hydriodic acid reduction of gliotoxin.[3]

When an intimate mixture of selenium and gliotoxin was heated at 230 to 250° a crystalline sublimate was obtained which analysis showed to have the formula $C_{12}H_8N_2O_3$. Sulfur, hydrogen sulfide and water were the only other products of the reaction which were observed, but under the conditions of the reaction carbon dioxide formaldehyde or methane might have been overlooked. Expressed in the form of an equation the reaction was

$$C_{12}H_{14}N_2O_4S_2 \xrightarrow[250^\circ]{Se} C_{12}H_8N_2O_3\ (II) + 2\,H_2S + [C] + H_2O$$

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

(2) Johnson, Bruce and Dutcher, THIS JOURNAL, **65**, 2005 (1943).

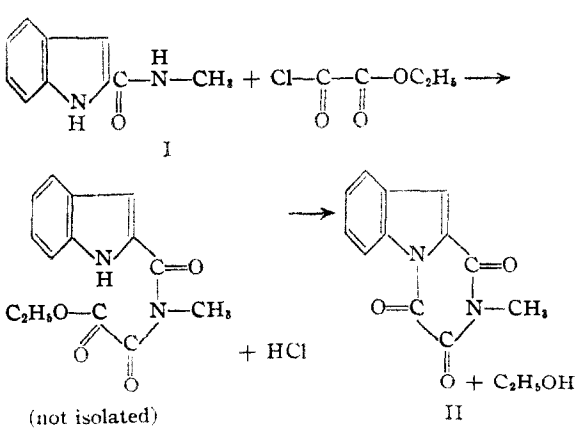
(3) Dutcher, Johnson and Bruce, *ibid.*, **66**, 617 (1944).

The structure of this product, II, was determined by means of alkaline hydrolysis. It was found to be split rapidly by methanolic potassium hydroxide at room temperature; approximately two moles of alkali were consumed. The product isolated from this hydrolysis was a neutral compound (I) possessing the formula $C_{10}H_{10}N_2O$. The equation for this reaction is

$$C_{12}H_8N_2O_3 + 2H_2O \xrightarrow[\text{methanol}]{KOH} C_{10}H_{10}N_2O(I) + H_2C_2O_4$$

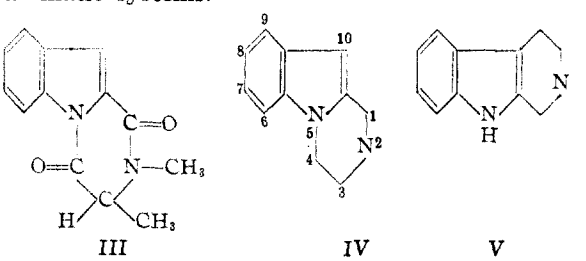
The neutral 10-carbon compound yielded indole-2-carboxylic acid and methylamine on vigorous hydrolysis with 25% aqueous potassium hydroxide. That I was the methyl amide of indole-2-carboxylic acid was shown by comparison with a synthetic sample. The other product of the original hydrolysis appeared from the equation to be oxalic acid although it was not isolated.

The structure assigned to the selenium degradation product is shown in formula II. This structure was proved by the synthesis of II from the methyl amide of indole-2-carboxylic acid and ethyl oxalochloride. When these two compounds were condensed in the presence of pyridine the product which was obtained was identical in all respects with the material obtained from gliotoxin by distillation with selenium. This synthesis is similar to that by which the hydriodic acid reduction product of gliotoxin, 2,3-dimethyl-1,4-diketotetrahydropyrazino[1,2-a]-indole (III), was prepared[3] and the reaction may be represented as follows



It has thus been possible to obtain by two different procedures degradation products of gliotoxin whose structures have been proved by synthesis. Both of these products contain the same tricyclic nucleus, IV, which according to the Ring Index No. 1630 is called pyrazino-[1.2-a]-indole. The selenium degradation product is therefore named 2-methyl-1,3,4-triketotetrahydropyrazino-[1.2-a]-indole. As in the synthesis of III,[3] the readiness with which the ring is opened and closed makes improbable the alternative mode of cyclization (V).

The action of selenium in splitting off a carbon atom from C_3 is difficult to explain in this particular case although it is well known that methyl groups are split off by this reagent in the dehydrogenation of sterols and polyterpenes to yield aromatic systems.[4]



We wish to thank the Cornell Research Foundation and E. I. du Pont de Nemours and Co. for generous support of this work.

Experimental

Selenium Distillation of Gliotoxin.—One gram of powdered gliotoxin was intimately ground with 5.0 g. of powdered black selenium. The gray mixture was placed in the bottom of a 50-ml. Erlenmeyer flask equipped with a short air condenser and heated in a metal bath. At approximately 200° water vapor and a yellow sublimate, apparently sulfur, began to come off. The mix was held at this temperature until all the water was off. The temperature was then allowed to rise gradually; at 230° a yellow, crystalline sublimate began to appear on the walls of the flask just above the surface of the melt. This product was collected over a period of one hour. When no more formed the temperature was raised to 250° but without further yield of product. About 50 mg. of crude sublimate was obtained. An additional 50 mg. of this compound was obtained by pulverizing the cooled melt in an iron mortar, extracting the powder in a Soxhlet with benzene and resubliming the benzene residue. Recrystallization of the combined sublimate from acetic acid gave rhombic plates with a pale yellow color, m. p. 253–255° with sublimation.[5]

Anal. Calcd. for $C_{12}H_8N_2O_3$: C, 63.20; H, 3.51; N, 12.30. Found: C, 63.40; H, 3.57; N, 12.38.

This product (II) was insoluble in water or dilute aqueous acid or alkali. It was insoluble in cold ethanol, methanol or ether and only slightly soluble in a large volume of hot ethanol. It was somewhat more soluble in acetone, benzene and chloroform, and dissolved readily in pyridine or dioxane. It was insoluble in concentrated hydrochloric acid but dissolved unchanged in concentrated sulfuric acid from which it was precipitated by the addition of water.

Alkaline Hydrolysis of the Selenium Sublimate.—A suspension of 75 mg. of the yellow crystals in 2.0 ml. of methanol was treated with 5.0 ml. of 1 *N* methanolic potassium hydroxide. The crystalline material dissolved on gentle warming. After standing for half an hour at room temperature the excess alkali was back-titrated with standard acid; 0.65 ml. of 1 *N* alkali had been consumed. Saponification equivalent found, 115; since the molecular weight for $C_{12}H_8N_2O_3$ is 228, two moles of alkali per mole of sublimate were used. The neutral solution was then concentrated in vacuum until all of the methanol had been removed. The colorless, crystalline precipitate (I) which had separated from the aqueous solution was filtered off, dried and recrystallized from benzene. Long, glistening

(4) L. Ruzicka and Thomann, *Helv. Chim. Acta*, **16**, 216 (1933); L. Ruzicka, Goldberg and Thomann, *ibid.*, **16**, 812 (1933); *ibid.*, **17**, 200 (1934); Harper, Kon and F. C. J. Ruzicka, *J. Chem. Soc.*, 124 (1934).

(5) All melting points are uncorrected.

needles were obtained which melted at 220° with sublimation. The yield was 55 mg. corresponding to 96% of the theoretical.

Anal. Calcd. for $C_{10}H_{10}N_2O$: C, 69.00; H, 5.75; N, 16.10. Found: C, 69.19; H, 5.89; N, 16.40.

Picrate.—The picrate of this neutral hydrolysis product was obtained by adding a solution of picric acid in benzene to a hot benzene solution of the compound. The picrate separated on cooling as long, deep red needles which melted at 168–170° with decomposition.

Anal. Calcd. for $C_{16}H_{13}N_5O_7$: N, 17.35. Found: N, 17.36.

Iodo Derivative.—The iodo derivative was easily prepared by adding a solution of iodine in potassium iodide to a dilute solution of the compound in 5% aqueous sodium hydroxide. It crystallized in clusters of long colorless needles, m. p. 186°. It was soluble in alcohol and acetone, insoluble in water, and quite stable under ordinary conditions.

Anal. Calcd. for $C_{10}H_9IN_2O$: I, 42.30. Found: I, 42.31.

Hydrolysis of Compound $C_{10}H_{10}N_2O$.—Forty mg. of the product of methanolic alkaline hydrolysis, $C_{10}H_{10}N_2O$ (I), was refluxed for two hours with a 25% aqueous solution of potassium hydroxide. Nitrogen was passed through the solution during the hydrolysis and the volatile base trapped in hydrochloric acid. This base was identified as monomethylamine by preparation of the hydrochloride and the chloroplatinate. From the alkaline solution indole-2-carboxylic acid was isolated in good yield. It melted at 204° and did not depress the melting point of an authentic sample.

Synthesis of Indole-2-carboxylic Acid Methyl Amide (I).—To confirm the identity of the neutral $C_{10}H_{10}N_2O$ product with indole-2-carboxylic acid N-methyl amide this compound was synthesized by the following two methods. 1. Indole-2-carboxylic acid chloride was prepared in the manner previously described[3] and was condensed in benzene solution with methylamine. Long, lustrous needles were obtained on recrystallization from benzene and melted at 219° with sublimation. No depression was observed in a mixed m. p. determination with the neutral product derived from gliotoxin. 2. The methyl amide was also obtained in good yield when indole-2-carboxylic acid ethyl ester reacted with a concentrated alcoholic solution of methylamine. The monomethyl amide of indole-2-carboxylic acid was readily soluble in ethanol, methanol and acetone; less soluble in cold benzene but soluble in hot benzene; it dissolved in hot water and crystallized out on cooling as lustrous, hexagonal platelets. It absorbed bromine in acetic acid solution to yield a crystalline bromo derivative, m. p. 185°. The iodo derivative was described above. The methyl amide coupled in alkaline solution with diazotized sulfanilic acid to form a deep orange-red solution; it gave a positive Ehrlich reaction (*p*-dimethylaminobenzaldehyde and concentrated hydrochloric acid).

Synthesis of the Selenium Degradation Product, $C_{12}H_8N_2O_3$ (II).—Although oxalic acid was not isolated from the hydrolysis products of the selenium sublimate, in view of the structure established for the remainder of the molecule, there was little doubt but that this dicarboxylic acid represented the missing fragment. This assumption was fully justified by the synthesis of the selenium degradation product (II) in the following manner.

Six hundred mg. of indole-2-carboxylic acid methyl amide (I) was dissolved in 10 ml. of dry pyridine and 25 ml. of dry ether added; to this solution was added slowly and with cooling a solution of 600 mg. of ethyl oxalochloride in 25 ml. of anhydrous ether. As the two solutions were mixed, a crystalline precipitate was formed which consisted partially of unchanged amide and partially of pyridine hydrochloride. The mixture was allowed to stand at room temperature for fifteen hours. The crystalline material was removed by filtration and the filtrate evaporated in vacuum. The sirupy residue crystallized upon the addition of ethanol; 115 mg. of pale yellow rhombs was obtained, m. p. 255°. The mother liquors contained some unchanged amide which was recovered and some non-crystalline oil, which probably represented uncyclized condensation products. The synthetic compound was identical in every respect with that isolated from gliotoxin. A mixed m. p. determination showed no depression.

Summary

The degradation of gliotoxin by selenium has been shown to yield 2-methyl-1,3,4-triketotetrahydropyrazino-[1.2-a]-indole. The structure of this substance has been determined by degradation and by synthesis. This formulation is in agreement with the structure of the product of the hydriodic acid reduction of gliotoxin previously published.

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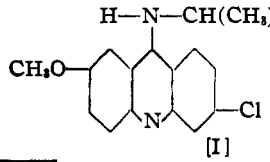
[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF IOWA STATE COLLEGE]

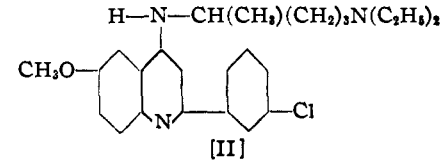
Some Quinolines Patterned as "Open Models" of Atabrine

BY HENRY GILMAN AND SYDNEY M. SPATZ[1]

It seemed of interest to prepare some so-called open models of atabrine [I] in connection with studies on attempted correlations of constitution with antimalarial action. One of these models is 6-methoxy-2-(3′-chlorophenyl)-4-[(α-methyl-δ-diethylaminobutyl)-amino]-quinoline [II]. This compound has a chlorophenyl group in place of the fused chlorobenzo group in atabrine.



[I]



[II]

Compound [II] was synthesized by the sequence of reactions shown beyond.

The over-all yield is quite satisfactory. *m*-Chlorophenyllithium was prepared in 70% yield

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