[CONTRIBUTION FROM THE SQUIBB INSTITUTE FOR MEDICAL RESEARCH]

Studies on the $C_{11}H_8N_2OS$ Degradation Product of Gliotoxin[1]

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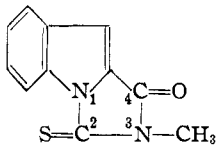
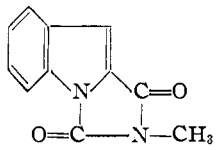
The conversion of the $C_{11}H_8N_2OS$ degradation product of gliotoxin to the corresponding $C_{11}H_8N_2O_2$ compound is described. The hydantoin nature of these products is deduced from hydrolysis studies and established by the synthesis of the $C_{11}H_8N_2O_2$ product through the condensation of ethyl indole-2-carboxylate with the sodium derivative of urethan followed by methylation with diazomethane. The location of the sulfur atom in the thiohydantoin is deduced from studies with the Ehrlich reaction and a tabulation of this reaction for numerous indole derivatives presented.

Although the thiohydantoin structure I was suggested for the $C_{11}H_8N_2OS$ product obtained from gliotoxin by alkaline hydrolysis[1] and further evidence for this structure adduced from its conversion to the hydantoin structure II,[3] conclusive proof, by unequivocal synthesis, was not achieved until recently.[4,5]



I



II

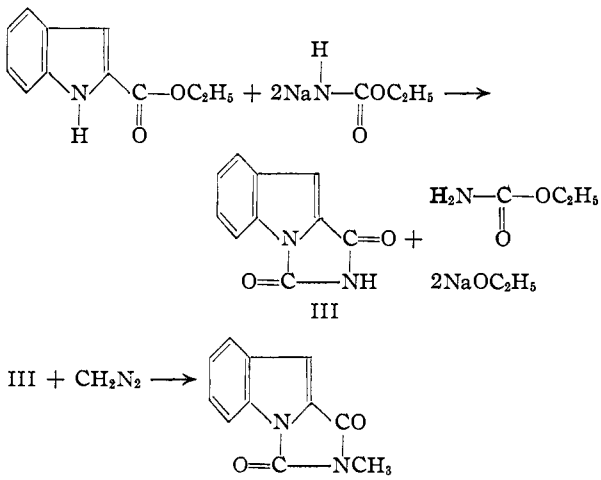
It is the purpose of this report to present the details of the transformation of the thiohydantoin to the normal hydantoin and to describe the synthesis of the latter as well as of several compounds of related structure which were prepared as possible intermediates for the synthesis of the thiohydantoin.

Chemical studies on the neutral C_{11}, sulfur containing, product had shown that it was not attacked during 2 hours refluxing in *N* methanolic potassium hydroxide nor acetylated by acetyl chloride in pyridine.[1] It could be desulfurized, however, with aluminum amalgam or zinc in hydrochloric acid and the reaction mixture gave a positive Ehrlich reaction which the original product did not. These observations were interpreted as evidence confirming structure I. The inability of the compound to be acetylated indicates cyclization through the indole nitrogen atom, and the conversion to a product giving a positive Ehrlich reaction is in accord with the placement of the sulfur atom on carbon 2 adjacent to the indole nitrogen, since, as may be seen from Fig. 1, none of the indole derivatives with C=O or C=S groups adjacent to the nitrogen atom gives the Ehrlich reaction, whereas if the substituting group is methyl or methylene, the reaction is positive. If the sulfur atom of the hydantoin were located on carbon 4 its removal with zinc and acid would still have left the C=O group at C_2 intact, and the Ehrlich reaction would have remained negative.

Treatment of the C_{11} product in pyridine solution with hydrogen peroxide at room temperature smoothly converted it into a product with the empirical formula $C_{11}H_8N_2O_2$, with melting point 181.5 to 182°. Confirmation of structure II for this neutral, sulfur-free compound was obtained by degradation and synthesis.

Through replacement of the sulfur atom with an oxygen atom the compound became more amenable to alkaline hydrolysis and when refluxed with 10% aqueous potassium hydroxide formed methylamine and indole-2-carboxylic acid in theoretical yield. Furthermore, treatment with 0.01 *N* aqueous potassium hydroxide at room temperature readily yielded indole-2-N-methyl-carboxamide.

The synthesis of the desired hydantoin was achieved by condensing ethyl indole-2-carboxylate with the sodium derivative of urethan and subsequently methylating the nitrogen atom of compound III with diazomethane as shown in the following scheme.



The synthetic product was identical in appearance, ultraviolet absorption spectrum and melting point with the product derived from gliotoxin.

Although the conditions under which the above condensation will take place were found to be very critical, no conditions could be found which would effect a similar condensation of ethyl indole-2-carboxylate with dithiourethan. The failure to synthesize the thiohydantoin I by this method as well as by the direct condensation of ethyl indole-2-carboxylate with methyl isothiocyanate, the method by which Elvidge and Spring[4] were subsequently able to accomplish its synthesis, led to a more extended study of the reactions of indole and its derivatives with various isocyanates and acyl isocyanates.

Although acyl isocyanates and acyl isothiocyanates will react with exceedingly weak bases such as

(1) J. D. Dutcher, J. R. Johnson and W. F. Bruce, THIS JOURNAL, **67**, 1736 (1945).

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(3) J. D. Dutcher, Abstracts of the 110th Meeting of the American Chemical Society, Chicago, Ill., September 9 to 13, 1946, 47B.

(4) J. A. Elvidge and F. S. Spring, *J. Chem. Soc.*, 135 (1949).

(5) J. R. Johnson and J. B. Buchanan, THIS JOURNAL, **73**, 3749 (1951).

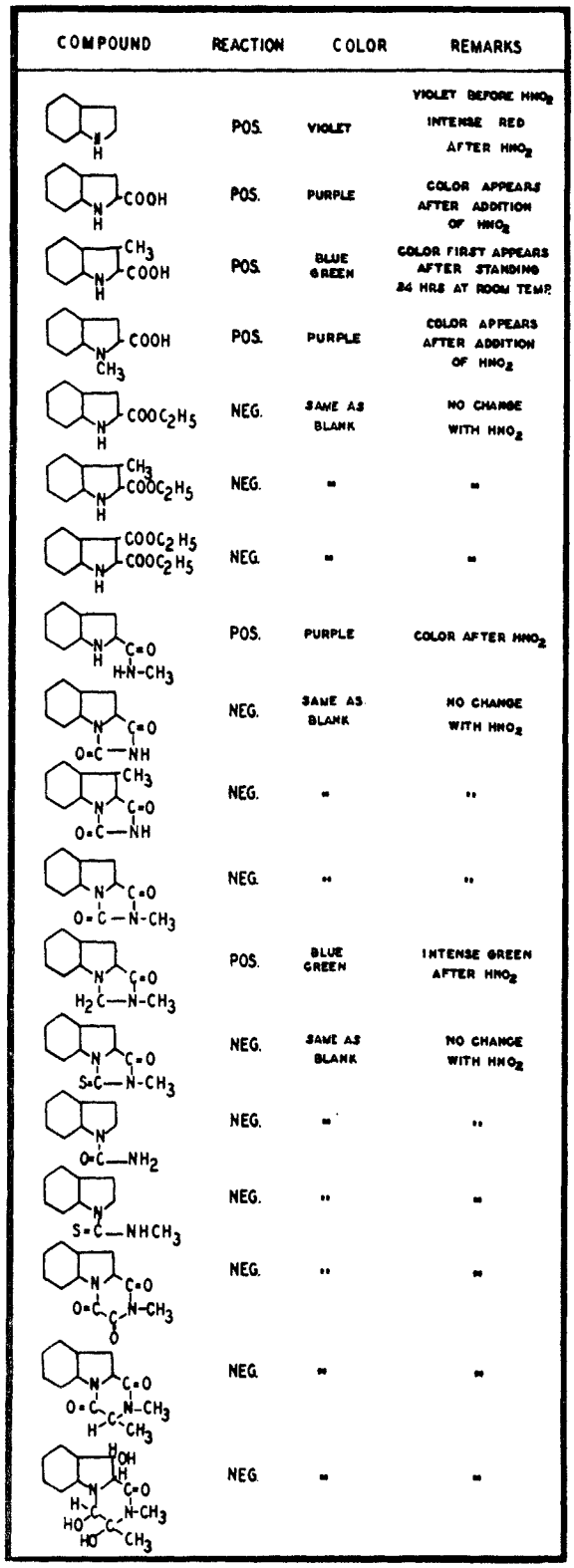
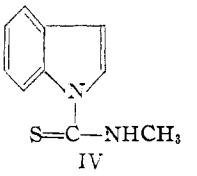


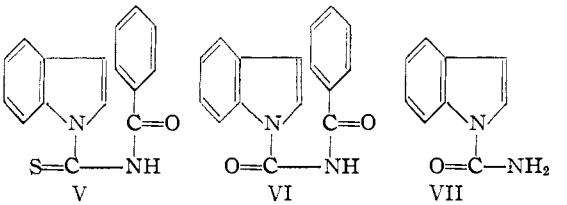
Fig. 1.—Color reactions of various indole derivatives with Ehrlich reagent. All the reactions were carried out in the following manner: The reagent, *p*-dimethylaminobenzaldehyde, was prepared as a 1% solution in 10% aqueous hydrochloric acid. The sample to be tested (0.5 to 1.0 mg.) was dissolved in 1.0 ml. of absolute ethanol and 0.1 ml. of reagent added. The mixture was immersed for 30 sec. in a boiling water-bath and the color noted; when cool, 2 drops of 10% aqueous $NaNO_2$ solution was added and the color again noted. The reaction is called positive when an intense blue or blue-violet color is produced. In some cases the color is only produced after the addition of HONO.

diphenylamine it was found that neither would react with indole-2-carboxylic acid or its esters under the various conditions employed. Interesting differences in reactivity were observed however when indole itself was used. A mixture of indole and methyl isocyanate was allowed to stand at room temperature for several weeks but no reaction occurred. Using methyl isothiocyanate, the mixture crystallized within a week with the formation of indole-1-N-methylthiocarbamide (IV).



IV

Both benzoyl isothiocyanate and benzoyl isocyanate react with indole but in this case the latter is the more reactive and the condensation is exothermic. The products of these reactions, V and VI, were subjected to mild hydrolysis but only VI gave an isolable product, the N-carbamidoindole (VII).



V VI VII

The ultraviolet absorption characteristics of these compounds are given in Table I. The similarity in shift in the absorption bands in going from the thio derivatives I, IV and V to their oxygen analogs II, VII and VI, respectively, may be noted.

TABLE I[a]

ULTRAVIOLET ABSORPTION CHARACTERISTICS OF COMPOUNDS I, II, IV, V, VI, VII

	$\lambda_{max.}$, mμ	log ε	$\lambda_{max.}$, mμ	log ε	$\lambda_{max.}$, mμ	log ε
Thio. compound, I	370	4.22	305	4.15	270	4.16
Oxy. compound, II[b]	310	4.16	255	4.01	235	4.13
Thio. compound, V	390	4.19	300	4.00	275	4.20
Oxy. compound, VI	305	4.12	265	4.06	240	4.31
Thio. compound, IV			307	4.01	260	4.28
Oxy. compound, VII			280	3.98	245	3.93

[a] Absorption spectra determined in ethanol solution. [b] These values are in agreement with those given by Elvidge and Spring.[4]

It is of interest also to tabulate the reaction with Ehrlich's *p*-dimethylaminobenzaldehyde reagent of the numerous indole derivatives encountered in the studies of gliotoxin. It may be seen in Fig. 1 that the reaction is negative when the indole-N atom is substituted by a C=O or C=S grouping even when both 2 and 3 positions are free. A carboxyl or carboxamide group at position 2 does not interfere but surprisingly enough the carbethoxy group does. Methyl or methylene groups at positions 1 and 3 do not prevent the reaction.

Experimental

Formation of the Hydantoin $C_{11}H_8N_2O_2$.—The thiohydantoin $C_{11}H_8N_2OS$, m.p. 188° (55 mg.), was dissolved in 3 ml. of pyridine and 0.5 ml. of 30% H_2O_2 added. The solution was allowed to stand at room temperature for 18 hours and then treated with water. The pale yellow crystalline product which separated out was centrifuged off and recrystallized from aqueous acetone solution after treating with a little Norit. Nearly colorless needles (30 mg.) were obtained which melted at 181.5 to 182°.[6,7] The ultraviolet absorption maxima are given in Table I.

Anal. Calcd. for $C_{11}H_8N_2O_2$: C, 66.00; H, 4.0; N, 14.00. Found: C, 66.01; H, 4.01; N, 13.92.

The aqueous pyridine supernatant solution gave an immediate precipitate of barium sulfate when tested with barium chloride solution.

Hydrolysis of the Hydantoin $C_{11}H_8N_2O_2$. (a) With 0.01 *N* KOH Solution.—A solution of 21.5 mg. in 16 ml. 0.01 *N* KOH, after standing 18 hr. at room temperature deposited a crop of colorless needles and concentration of the neutralized solution yielded a second crop; total yield 17 mg. After recrystallization from benzene, the long colorless needles had m.p. 221° and gave no depression in a mixed melting point determination with an authentic sample of indole-2-N-methylcarboxamide, m.p. 220°.[8]

(b) With 10% KOH solution.—During 3 hr. of refluxing in 10% KOH solution, 20 mg. (0.1 mmole) of the hydantoin, yielded exactly 0.1 mmole of methylamine by aeration into standard acid solution. Acidification of the aqueous solution, followed by extraction with chloroform and evaporation of the solvent yielded 16 mg. of crystalline indole-2-carboxylic acid, m.p. 204°.

Synthesis of the Hydantoin, $C_{11}H_8N_2O_2$. (a) Condensation of Ethyl Indole-2-carboxylate and Urethan.—After several unsuccessful attempts to condense indole-2-carboxylic acid or the acid chloride[9] with urethan in various solvents, including benzene, pyridine and isopropyl ether, the following procedure was found to bring about the desired reaction. Three equivalents, 292.5 mg. of metallic sodium were treated in 17 ml. of anhydrous ether with 3.5 equivalents, 1300 mg. of dry urethan. After two hours gentle warming at 40° the solution was allowed to stand at room temperature overnight protected by a calcium chloride tube. At this point one equivalent, 739 mg. of ethyl indole-2-carboxylate in 15 ml. ether was added and the solution refluxed for 8 hr. Initially the solution turned intensely green but gradually lightened to a pale yellow-green color. After standing at room temperature overnight the reaction mixture was worked up by adding water to dissolve the precipitate which had formed and then separating the ethereal and aqueous phases. Each phase was washed once with the corresponding solvent. The ether was dried over Na_2SO_4 and evaporated *in vacuo* to yield 190 mg. of unreacted ester. The aqueous solution was acidified to *p*H 4 and the gray, voluminous precipitate centrifuged down, washed with water and crystallized from 95% ethanol. Tan colored needles (440 mg.) were obtained with melting point 250–260°. Recrystallization from acetone containing a small amount of water and treatment with a little Darco G60 yielded 245 mg. of colorless, glistening platelets with melting point 263–264° with softening and sublimation beginning about 240°.

Anal. Calcd. for $C_{10}H_6N_2O_2$: C, 64.51; H, 3.25; N, 15.05. Found: C, 64.21, 64.65; H, 3.55, 2.94; N, 15.42, 14.91.

Similar runs using metallic potassium instead of sodium did not yield any of the desired hydantoin, but repeat runs under conditions identical with the above always gave a similar yield of product.

(6) All melting points uncorrected.

(7) Elvidge and Spring[4] also prepared this compound from their synthetic thiohydantoin by oxidation with H_2O_2 in ethanol solution. They report the melting point as 182°.

(8) J. D. Dutcher, J. R. Johnson and W. F. Bruce, THIS JOURNAL, **66**, 619 (1944).

(9) J. R. Johnson, R. B. Hasbrouck, J. D. Dutcher and W. F. Bruce, *ibid.*, **67**, 423 (1945).

(b) Methylation.—No methylation product could be obtained using methyl iodide and methanolic KOH, presumably because of the rapid hydrolysis of the hydantoin. However, when 242 mg. were dissolved in dry acetone and treated with approximately 500 mg. of diazomethane in dry ether a brisk evolution of nitrogen occurred and the solution yielded on evaporation pale yellow needles of m.p. 180°. Recrystallization from aqueous acetone (1:1) yielded 135 mg. of long silky needles which melted at 181.5 to 182° and gave no depression in a mixed melting point determination with the hydantoin derived from gliotoxin The ultraviolet absorption spectra were identical.

Anal. Calcd. for $C_{11}H_8O_2N_2$: C, 66.00; H, 4.0; N, 14.00. Found: C, 65.93; H, 4.3; N, 14.17.

N-Methyl-thio-1-indolecarboxamide (Indole-1-N-methylthiocarboxamide) (Compound IV).—A mixture of 3.0 g. of indole and 2.5 g. of methyl isothiocyanate in 8.0 ml. of benzene was allowed to stand at room temperature. After one week the solution had set to a crystalline mass. The crystals were filtered off, washed repeatedly with hexane and recrystallized from ethanol–hexane mixture. The crystalline product, with melting point 75 to 76° after softening at 74° is very soluble in ethanol and methanol, less soluble in benzene or hexane, nearly insoluble in cold water.

Anal. Calcd. for $C_{10}H_{10}N_2S$: C, 63.20; H, 5.26. Found: C, 62.95; H, 5.11.

The ultraviolet absorption maxima are given in Table I; the Ehrlich reaction is negative.

N-Benzoylthio-1-indolecarboxamide (Indole-1-N-benzoylcarboxamide) (Compound V).—When a benzene solution containing 520 mg. of benzoyl isothiocyanate (prepared from benzoyl chloride and lead thiocyanate) was added to a solution of 371 mg. of indole in 5 ml. dry benzene the mixture became deep orange in color within 3 hr. After standing at room temperature for 3 days the solution was concentrated *in vacuo* to remove the benzene. The orange-brown sirupy residue was washed repeatedly with hot hexane to remove unreacted indole and benzoyl isothiocyanate. The residue slowly crystallized. The crystals were separated from the sirupy mother liquor with benzene. The product was very insoluble in most organic solvents but could be recrystallized from a large volume of hot dioxane. One hundred mg. of shiny orange platelets with melting point 169 to 171° with decomposition was obtained.

Anal. Calcd. for $C_{16}H_{12}N_2OS$: C, 68.50; H, 4.28; S, 11.41. Found: C, 68.23; H, 4.58; S, 11.28.

The ultraviolet absorption maxima measured in dilute ethanol solution are given in Table I.

N-Benzoyl-1-indolecarboxamide (Indole-1-N-benzoylcarboxamide) (Compound VI).—When indole and benzoyl isocyanate were mixed in 1:1 molar ratio the solution warmed spontaneously and crystalline material soon formed. After the reaction had cooled the solid was washed with benzene and the crystals filtered and dried. The nearly colorless crystals can be recrystallized from hot ethanol; they melt at 234–236°.

Anal. Calcd. for $C_{16}H_{12}N_2O_2$: C, 72.70; H, 4.55; N. 10.60. Found: C, 72.86; H, 5.00; N, 10.87.

The ultraviolet absorption bands are listed in Table I.

Indole-1-carboxamide (Compound VII).—Hydrolysis with 5 ml. of 1.0 *N* aqueous NaOH solution of 510 mg. of Compound VI by gentle warming for 1 hr. on the steam-bath yielded two products in about equal amount. When the alkaline solution cooled a crop of colorless platelets crystallized out, weight 171 mg., melting point 198–199°. It was recrystallized for analysis from hot water.

Anal. Calcd. for $C_9H_8N_2O$: C, 67.50; H, 5.00; N, 17.50. Found: C, 67.58; H, 5.08; N, 17.78.

The ultraviolet absorption bands are listed in Table I. The Ehrlich reaction is negative.

Neutralization of the alkaline solution from the above hydrolysis yielded 150 mg. of a crystalline precipitate with melting point at 128° which was identified as benzamide (m.p. 128°).

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