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Indole-3-acetic acids and 2-carboxyindole-3-acetic acids with substituents in the benzene ring

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NOTE TO USERS

INDOLE-3-ACETIC ACIDS AND 2-CARBOXYINDOLE-3-ACETIC ACIDS WITH SUBSTITUENTS IN THE BENZENE RING

by

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Dean of Graduate College

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INTRODUCTION

The substituted indole-3-acetic acids are worthy of investigation as possible indole-3-acetic acid antagonists and as more difficultly catabolizable analogs of indole-3-acetic acid. Previous work in these laboratories (1, 2) has shown that the chloro-substituted 2-methylindole-3-acetic acids are more active in the Went Pea Test than the parent 2-methylindole-3-acetic acid. Of the chloro-substituted compounds prepared the 5-chloro was more active than the 7-isomer, which was in turn more active than the 5,7-dichloro-2-methylindole-3-acetic acid. In so far as this is a fair comparison, the order of decreasing activity did not parallel the activities found by Zimmerman and Hitchcock (3) for the chloro-substituted phenoxyacetic acids where it was observed that the order of decreasing activity was in the order 2,4-dichlorophenoxyacetic acid, p-chlorophenoxyacetic acid, o-chlorophenoxyacetic acid. However, in both series of compounds the activity was increased by the substitution of chlorine in the benzene ring. In the light of these results it was of interest to evaluate the activity of the indole-3-

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(1) Stevens, Ph.D. Thesis, Iowa State College, 1947.
(2) Stevens and Fox, J. Am. Chem. Soc., 70, 2263 (1948).
(3) Zimmerman and Hitchcock, Contrib. Boyce Thompson Inst., 12, 321 (1941-2).

acetic acids with chlorine substituted in the benzene ring.

The substituted 2-carboxyindole-3-acetic acids are of interest on their own merits as well as for their use as possible intermediates in an alternate method (4, 5) of synthesis of the corresponding indole-3-acetic acids by partial decarboxylation of the 2-carboxy derivatives.

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(4) Findlay and Dougherty, J. Org. Chem., 13, 560 (1948).
(5) King and L' Ecuyer, J. Chem. Soc., 1901-5 (1934).

HISTORICAL

The history of the naturally occurring plant growth hormones has been reviewed by Stevens (1). Stevens has also discussed in detail the known analogs of indole-3-acetic acid as well as the mechanisms which had been proposed for the Fischer indole synthesis up to 1947. Since that time an additional synthesis of indole-3-acetic acid has been developed (6). This method involves the reaction between gramine and sodium cyanide to give a mixture of indole-3-acetamide and indole-3-acetic acid.

Several substituted indole-3-acetic acids have been prepared since Stevens' review. The 1-methylindole-3-acetic acid has been synthesized by treatment of the methiodide of 1-methylgramine with sodium cyanide to give 1-methylindole-3-acetonitrile, which was hydrolyzed to 1-methylindole-3-acetic acid (7).

Findlay and Dougherty (4) have prepared some of the methoxyindole-3-acetic acids and methoxy-2-carboxyindole-3-acetic acids. These workers used the procedure developed by King and L'Ecuyer (5) and Tanaka (8) for the synthesis of indole-3-acetic acid. The ethyl α-ketoglutarate

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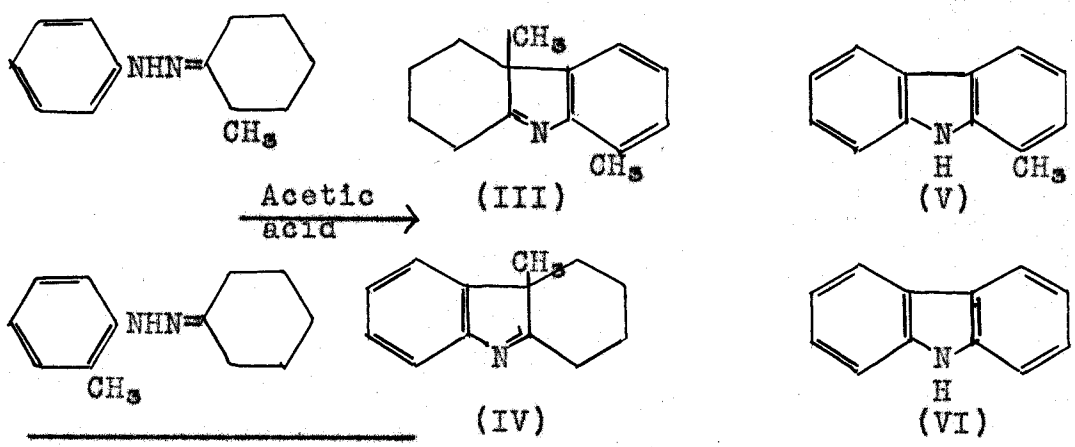
(6) Snyder and Pilgrim, J. Am. Chem. Soc., 70, 3770 (1948).
(7) Snyder and Eliel, J. Am. Chem. Soc., 70, 1703-5 (1948).
(8) Tanaka, J. Pharm. Soc. Japan, 60, 74-6 (1940).

methoxyphenylhydrazones were prepared from ethyl α-acetoglutarate and the methoxyphenyldiazonium chloride by the Japp-Klingemann reaction. The phenylhydrazones were then cyclized to the corresponding 2-carboxyindole-3-acetic acid derivative. The 5-, 6-, and 7-methoxy- and 5,6-dimethoxy-2-carboxyindole-3-acetic acids were prepared by this procedure. The 6-methoxy-2-carboxyindole-3-acetic acid was obtained by cyclization of ethyl α-ketoglutarate m-methoxyphenylhydrazone. Since cyclization of a m-substituted phenylhydrazone would be expected to yield a mixture of the 4- and 6-isomers and since no evidence was offered to prove the structure of their product, the identity of the acid is not established. Work in these laboratories on an analogous reaction, cyclization of succinaldehydic acid m-chlorophenylhydrazone, has shown that two isomers are formed as expected. In the case of the 4- and 6-chloroindole-3-acetic acids an eutectic mixture was formed which had the properties of a pure compound. It seems possible, in the light of these results, that the 6-methoxy-2-carboxyindole-3-acetic acid obtained by Findlay and Dougherty was a mixture of the 4- and 6-methoxy derivatives. At any rate the formation of the 6-methoxy derivative as the sole product should be considered open to question. No satisfactory analysis was obtained for the 5,6-dimethoxy-2-carboxyindole-3-acetic acid, and this acid could not be decarboxylated to the 5,6-dimethoxyindole-

3-acetic acid. However, the 5-, 6(?)-, and 7-methoxy-2-carboxyindole-3-acetic acids were successfully decarboxylated to the corresponding indole-3-acetic acids.

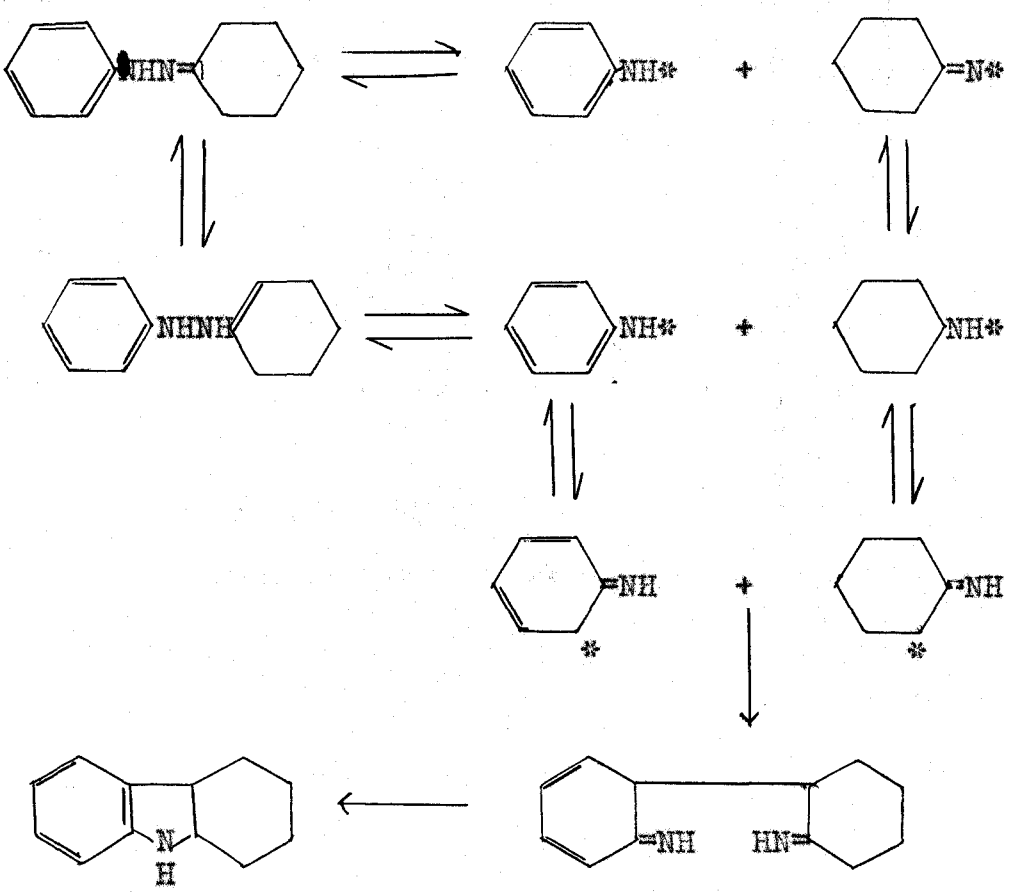
The activities of these methoxyindole-3-acetic acids in the Went Pea Test was of the same order of magnitude as the activity of indole-3-acetic acid (9).

Recent studies on the mechanism of the Fischer indole synthesis include the work of Pausacker and co-workers (10-14). These workers found that cyclizing a mixture of cyclohexanone o-tolylphenylhydrazone and 2-methylcyclohexanone phenylhydrazone gave, after dehydrogenation of the neutral fraction, not only the expected 1-methylcarbazole (V) and 11-methyl-1,2,3,4-tetrahydrocarbazolenine (IV) but also an appreciable amount of 8,11-di-



(9) Findlay and Dougherty, J. Biol. Chem., 183, 361 (1950).
(10) Barnes, Pausacker and Schubert, J. Chem. Soc., 1381-4 (1949).
(11) Pausacker and Schubert, J. Chem. Soc., 1384-9 (1949).
(12) Pausacker and Schubert, Nature, 163, 289 (1949).
(13) Pausacker and Schubert, Nature, 163, 602 (1949).
(14) Pausacker, J. Chem. Soc., 621-4 (1950).

methyl-1,2,3,4-tetrahydrocarbazolenine (III) and a small amount of carbazole (VI). In the light of this and similar evidence they concluded that the reaction was intermolecular and proposed the following free radical mechanism for the Fischer indole synthesis:



Gore, Hughes and Ritchie (15) have pointed out that if the free radical mechanism were correct some 1,8-dimethylcarbazole should have been found in the dehydro-

(15) Gore, Huges, and Ritchie, Nature, 164, 835 (1949).

genated tetrahydrocarbazole fraction. None of this product was found. These workers have also stated that some of the C_6H_5NH* radicals should combine to form hydrazobenzene which under the reaction conditions would be converted to benzidine. Tests sensitive to 0.0003 g. of benzidine gave negative results. The free radicals formed by the dissociation of the phenylhydrazone would react mainly with the solvent to form a large amount of carbon dioxide.

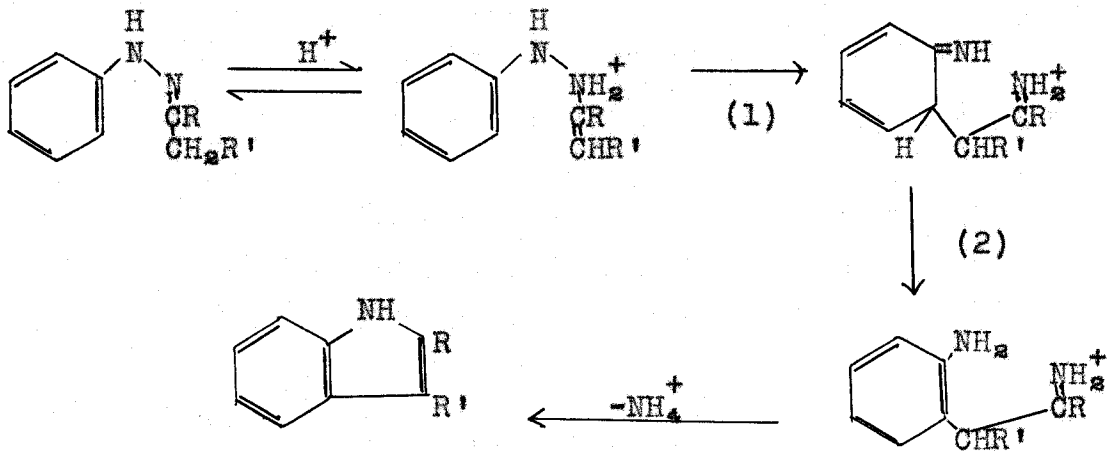
$$X* + CH_3COOH \longrightarrow CH_3COO* + HX$$
$$\downarrow$$
$$CH_3* + CO_2$$

The yield of CO_2 from the cyclization of cyclohexanone phenylhydrazone was only 0.4%. These arguments discredit any intermolecular free radical mechanism. Gore, Hughes and Ritchie have proved beyond any reasonable doubt that the products obtained in the cyclization of a mixture of phenylhydrazones can be explained by an exchange reaction between the two phenylhydrazones before cyclization occurs. A mixture of two phenylhydrazones in boiling glacial

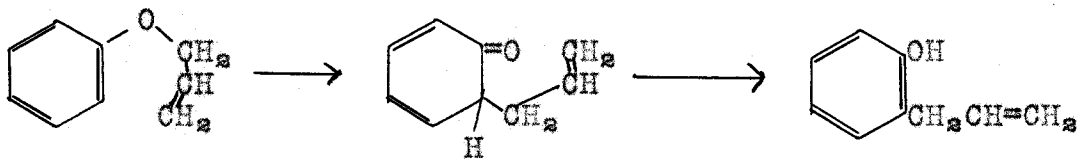
$$C_6H_5NHN{=}CHR + XC_6H_4NHN{=}CHR' \rightleftharpoons C_6H_5NHN{=}CHR' + XC_6H_4NHN{=}CHR$$

acetic acid then consists of four phenylhydrazones, any of which can cyclize to the carbazole or indole as the constitution of the hydrazone permits.

Carlin and Fisher (16) have proposed an entirely different mechanism for the Fischer cyclization. If the mechanism proposed by the Robinsons (17) for the Fischer indole synthesis is written in the following manner it is clear that a formal analogy exists between steps 1



and 2 and the two steps which have been proposed for the Claisen rearrangement to the ortho position (18).



Investigations of the Claisen rearrangement have revealed that 2,6-disubstituted phenyl allyl ethers, in which the ortho position is blocked often undergo para

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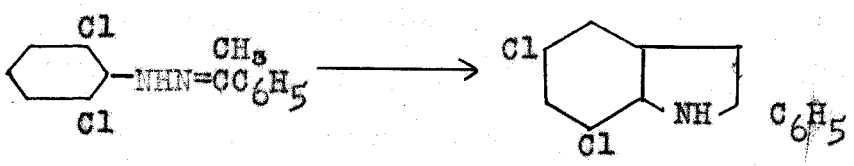
(16) Carlin and Fisher, J. Am. Chem. Soc., 70, 3421 (1948).
(17) Robinson and Robinson, J. Chem. Soc., 113, 639 (1918), 125, 827 (1924).
(18) Tarbell, "Organic Reactions" Vol. II, John Wiley and Sons, Inc., New York, N.Y., 1944, p. 16.

migration of the allylic group although the mechanism of the para rearrangement is apparently different from that of the ortho rearrangement (19). Certain 2,6-dibromo (20) and 2,6-dichlorophenyl allyl ethers (21) have been shown to undergo both ortho rearrangement, with displacement of a halogen atom, and para rearrangement.

If a 2,6-disubstituted phenylhydrazone were subjected to cyclization the rearrangement could go either

(1) ortho with displacement of the corresponding atom or group or

(2) para to give aldehydes which would be converted to tars under the reaction conditions. Experiments on several phenylhydrazones in this 2,6-disubstituted class gave large amounts of intractable tars and low yields (7 to 25%) of unexpected 5,7-disubstituted indoles. A typical example is given:



This reaction, in which a halogen apparently migrates from an ortho to the para position, has no recorded analog among either the benzidine or Claisen

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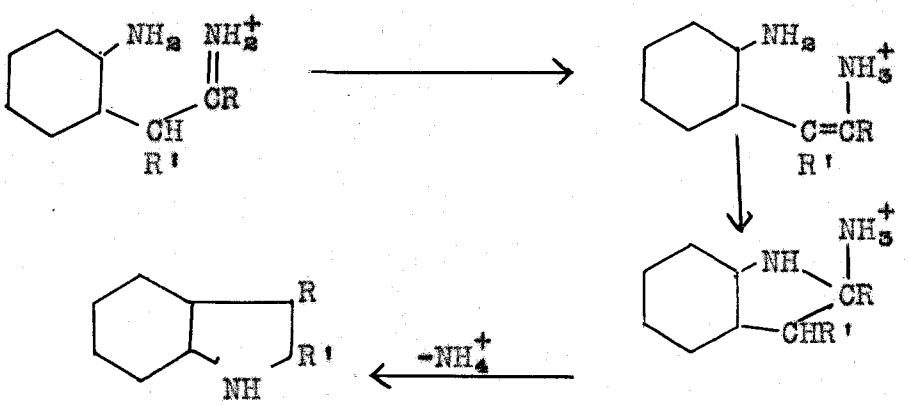
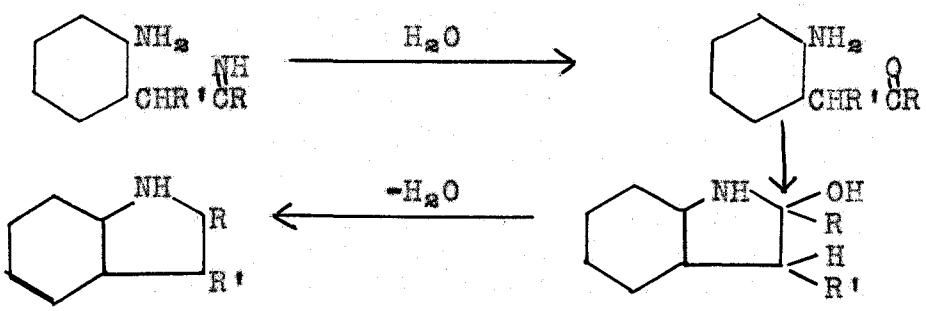
(19) Tarbell, Chem. Rev., 27, 495 (1940).
(20) Hurd and Webb, J. Am. Chem. Soc., 58, 2190 (1936).
(21) Tarbell and Wilson, J. Am. Chem. Soc., 64, 1066 (1942).

rearrangements.

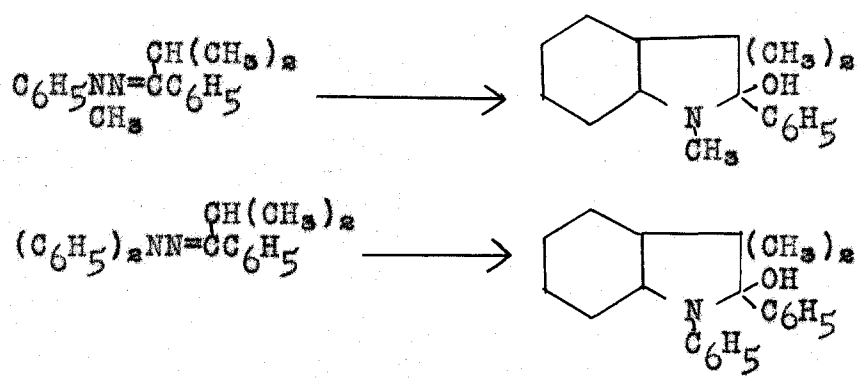
While Carlin and Fisher were not able to prove that the Fischer indole synthesis is analogous to the Claisen rearrangement, their proposed mechanism explains the absence of any para rearrangement product. The latter product would be expected if the true course of the reaction involved an orthobenzidine rearrangement. The last step in the reaction can proceed by two different paths. The first, which probably represents the true course of the reaction in most syntheses, is shown below.



A second route would involve hydrolysis of the imine to a carbonyl. The ring would then be closed by the loss of water in a two-step process.



This second alternative can not occur in cyclizations with anhydrous zinc chloride or with boron triflouride with no solvent or with dry benzene as solvent. However, some cyclizations are best explained on this basis. Two examples are the work of Jenisch (22) who reported that cyclization of the α-methyl-α-phenylhydrazone of isopropyl phenyl ketone gave 1,3,3-trimethyl-2-phenyl-2-hydroxyindole, and of Neber et al. (23) who obtained 1,2-diphenyl-2-hydroxy-3,3-dimethylindole from the cyclization of the α,α-diphenylhydrazone of phenyl isopropyl ketone.



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(22) Jenisch, Monatsh., 27, 1223 (1906).
(23) Neber, Gertrud, Knoller, Herbst and Trissler, Ann., 471, 113 (1929).

EXPERIMENTAL*

Preparation of Indole-3-acetic Acid

Succinaldehydic acid phenylhydrazone

This compound was prepared by Stevens' (1) procedure. To 24.9 g. (0.20 mole) of glutamic acid in 400 ml. of 0.5 M sodium hydroxide solution, 200 ml. of 1 M sodium hypochlorite (24) solution was added. The solution was stirred until a negative starch-iodide test was obtained and then acidified by the addition of 70 ml. of 3N hydrochloric acid. Carbon dioxide was evolved during the acidification. The solution was heated to 50° and maintained at that temperature until a negative starch-iodide test was obtained (50 minutes). A solution of 22.0 g. (0.20 mole) of phenylhydrazine in 50 ml. of 25% acetic acid was added and the reaction mixture heated for twenty minutes on the steam bath. The reaction mixture was acidified to Congo red with hydrochloric acid, cooled and extracted once with 200 ml. of ether and once with

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(24) Prepared by the method of Raschig, Ber., 40, 4580 (1907). A commercial solution of sodium hypochlorite, "Chlorox", was found to be unsatisfactory.

*Melting points are uncorrected. Nitrogen determinations were done by the micro Dumas method, and the chlorine analysis by a Volhard titration after the sample was burned in a Parr bomb.

100 ml. of ether. The combined ether extracts were dried over sodium sulfate and distilled. This yielded 18 g., 47%, of crude succinaldehydic acid phenylhydrazone.

Indole-3-acetic acid

Sixteen grams (0.0831 mole) of crude succinaldehydic acid phenylhydrazone were refluxed five hours, under nitrogen, with a solution of 20 ml. of concentrated sulfuric acid in 180 ml. of commercial absolute ethanol. The cooled solution was poured into a liter of water and the oil which separated was extracted with three 200 ml. portions of ether. The combined ether extracts were dried over sodium sulfate containing a small amount of potassium carbonate to neutralize any acid in the ether. Distillation of the ether and alcohol left 12.7 g. of an orange oil.

The crude ethyl indole-3-acetate was purified by vacuum distillation. The fraction distilling 160-180° at 0.2 mm., wt. 7.2 g., was collected as product. A second fraction distilling 180-220° at 0.2 mm. weighed 1.5 g. Saponification of this second fraction gave 0.85 g. of 2-carboxyindole-3-acetic acid, which melted 231-233° (dec.) after two recrystallizations from water.

Anal. Calcd. for $C_{11}H_9O_4N$: Neut. equiv., 109.5; N, 6.40

Found: Neut. equiv., 110 (potentiometric);
N, 6.32, 6.34

A mixed melting point with an authentic sample of 2-carboxyindole-3-acetic acid, m.p. 236° (dec.), prepared by the method of King and L'Ecuyer (5) was 235-236° (dec.).

The principal product was saponified by refluxing one hour with 100 ml. of 10% methanolic potassium hydroxide. The solution was diluted with 200 ml. of water and distilled until the temperature of the vapor reached 99°. The alkaline solution was extracted once with 50 ml. of ether. Most of the ether was distilled out of the aqueous layer by reducing the pressure with an aspirator. Acidification of the aqueous solution slowly with 10% hydrochloric acid gave a light brown crystalline product. The crystals were filtered off, washed three times with water and air dried in the filter. The product was washed with a small amount of chloroform and dried. The product was recrystallized from water (Norit A). This gave 1.8 g. of indole-3-acetic acid, m.p. 164-165° (dec.). A mixed melting point with an authentic sample of indole-3-acetic acid showed no depression. The mother liquors were combined and extracted with three 30 ml. portions of ether. The combined ether extracts were dried over sodium sulfate and distilled.

The residue was extracted with 10 ml. of chloroform. The crystals were filtered off and washed with three 5 ml. portions of chloroform. This gave 1.5 g. of product, m.p. 155-160°. After one recrystallization from 20 ml. of water this product melted 165-167° (dec.), and weighed 1.2 g. The total yield of pure indole-3-acetic acid was 3.0 g., 21%.

The results of some other runs are summarized in Table I.

Table I

Yields of Indole-3-acetic Acid in Several Runs

Run No.	Grams of crude succinaldehydic acid phenylhydrazone	Cyclization agent	Yield of crude product %	Yield of pure indole-3-acetic acid %
1	28.0	70 ml. H_2SO_4 in 700 ml. of ethanol	34	28[a]
2	9.0	10 ml. H_2SO_4 in 90 ml. of ethanol	32	19[b]
3	8.2	10 ml. H_2SO_4 in 90 ml. of ethanol	32	24[b]

[a]The ester was distilled in a molecular still.

[b]The ester was distilled through a short vacuum-jacketed Claisen still head.

Attempts to cyclize succinaldehydic acid phenylhydrazone with boron trifluoride without solvent and with

acetic acid as solvent were not successful. An attempt to cyclize the ethyl ester of the succinaldehydic acid phenylhydrazone with boron trifluoride was also unsuccessful, as was an attempt to cyclize the acid with hydrochloric acid solution saturated with zinc chloride. Since no starting material was isolated in any of the unsuccessful experiments, it is not known whether cyclization failed or whether the negative results were due to the difficulty in isolating the product from the reaction mixture.

Preparation of 2-Carboxyindole-3-acetic Acid

The ethyl α-acetoglutarate was prepared according to the procedure described in Organic Syntheses (25). The remainder of the synthesis is patterned after the work of King and L'Ecuyer (5).

Ethyl α-acetoglutarate

A solution of sodium ethoxide was prepared by dissolving 2.52 g. (0.11 mole) of sodium in 40 ml. of anhydrous ethanol, and 15.8 g. (0.12 mole) of acetoacetic ester were added at a rate of 100 drops per minute. The solution was heated at reflux and stirred mechanically while 20 g. (0.11 mole) of ethyl β-bromopropionate was added at 100 drops per minute. Sodium bromide began

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(25) Adkins, Isbel and Wojcik, Org. Syntheses, Coll. Vol. 2, 263 (1943).

separating immediately. The suspension was refluxed for six hours and allowed to cool. The sodium bromide was filtered off. After distillation of the ethanol at atmospheric pressure the product was vacuum distilled. The fraction boiling 143-149° at 5.5 mm. was collected as the product. The yield was 18.0 g. (0.0781 mole), 71%.

Ethyl α-ketoglutarate phenylhydrazone

Six grams (0.026 mole) of ethyl α-acetoglutarate was dissolved in 25 ml. of ethanol. The solution was cooled to approximately -20° and 18.5 ml. of cold aqueous 20% sodium hydroxide added. Immediately a solution of benzenediazonium chloride, prepared from 2.5 g. (0.027 mole) of aniline, 1.9 g. (0.0275 mole) of sodium nitrite, 7.6 ml. of concentrated hydrochloric acid and 13 ml. of water, was added. An orange oil separated immediately. The solution was shaken for 10 minutes and allowed to warm up to room temperature. The solution was again cooled and acidified with 3 N hydrochloric acid. The oil did not crystallize so the solution was diluted with 150 ml. of water, and the oil recovered by extraction with two small protions of ether. The combined ether extracts were dried over sodium sulfate and distilled. The oily residue was used without further purification

for the next synthesis.

2-Carboxyindole-3-acetic acid

The crude phenylhydrazone, prepared above, was cyclized by dissolving in 20 ml. of absolute ethanol and refluxing fifty minutes while a rapid stream of hydrogen chloride was bubbled through the solution. A solid (NH_4Cl) separated during the cyclization and the solution became quite dark. The cooled reaction mixture was poured into 100 ml. of water and the oil, which did not crystallize, was extracted with three 50 ml. portions of ether. The combined, wet ether extracts were distilled, and the residue was saponified by refluxing twenty minutes with 50 ml. of 10% ethanolic sodium hydroxide. The reaction mixture was cooled; the insoluble disodium salt was filtered off and washed with ethanol. The salt, still wet with ethanol, was dissolved in 20 ml. of water and the acid precipitated by acidification with 3 N hydrochloric acid. This gave 2.9 g. (0.133 mole) of well formed crystals, m.p. 233° (dec.). This represents a yield of 51% based on the ethyl α-acetoglutarate used. After one recrystallization from 10% ethanol (decolorizing charcoal) the acid melted 236° (dec.).

A mixed melting point with the product obtained from the cyclization of crude succinaldehydic acid phenyl-

hydrazone showed no depression.

Attempted Preparation of 5-Chloroindole-2-carboxylic Acid

Pyruvic acid p-chlorophenylhydrazone

This compound was prepared by the method of Hewitt (26). Forty-five grams (0.251 mole) of p-chlorophenylhydrazine hydrochloride were dissolved in 1 liter of water at 40°. Ten ml. of 3 N hydrochloric acid were added in an attempt to remove a slight turbidity, and a solution of 20 ml. (0.30 mole) of pyruvic acid (Matheson Co., Inc.) in 150 ml. of water was added to the rapidly stirred solution. A voluminous precipitate formed immediately. After stirring for ninety minutes the product was filtered off, washed with very dilute hydrochloric acid and pressed free of excess solvent. The dried material weighed 47 g. (0.222 mole), 89%. The product began softening at 186° and decomposed 191-192°. Recrystallization of the product from an ethanol-water mixture did not raise the melting point. Hewitt gives the melting point as 199°; however, the compound was sufficiently pure for the next experiment.

Attempted cyclization of pyruvic acid p-chlorophenylhydrazone

The procedures described in the literature for the

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(26) Hewitt, J. Chem. Soc., 63, 873 (1893).

preparation of indole-2-carboxylic acid from pyruvic acid phenylhydrazone with zinc chloride (27) and boron trifluoride (28) give yields of less than eight per cent. However, Robinson (29) describes a method for cyclizing pyruvic acid p-tolylhydrazone with ethanolic HCl in a yield of 60%. This method was attempted here.

Thirty grams (0.16 mole) of pyruvic acid p-chlorophenylhydrazone were dissolved in 400 ml. of absolute ethanol and a rapid stream of dry hydrogen chloride was bubbled through the refluxing solution for four hours. No ammonium chloride separated, indicating that no cyclization had occurred.

Eighty-five ml. of concentrated sulfuric acid was added and the solution heated to reflux. The hydrogen chloride distilled out rapidly. It was noticed that a colorless, odorless, combustible gas was slowly evolved at this temperature. The temperature of the solution was lowered to 65-70° where there was no evolution of gas and then maintained at that temperature for four hours. The cooled reaction mixture was poured into 1 liter of water and the aqueous suspension extracted repeatedly with ether. Distillation of the combined, dried (sodium sul-

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(27) Fischer, Ann. 236, 116 (1886); Ber., 19, 1563 (1886); 21, 1071, 1811 (1888).
(28) Snyder and Smith, J. Am. Chem. Soc., 65, 2452 (1943).
(29) Robinson, J. Biol. Chem., 62, 495 (1924).

fate) ether extracts gave 17 g. of a black tar from which no product could be isolated. However, despite this failure, this cyclization has been reported by Rydon (30) who gave no experimental details.

Preparation of 5-Chloroindole-3-acetic Acid

Succinaldehydic acid p-chlorophenylhydrazone

A solution of 0.2 mole of sodium hypochlorite in 202.5 ml. of solution was added to a mechanically stirred solution of 32.3 g. (0.22 mole) of glutamic acid in 400 ml. of 0.5 M sodium hydroxide. The solution was heated to 50° until a negative starch-iodide test was obtained. The solution was acidified with 10% hydrochloric acid and ten minutes later a solution of 37 g. (0.20 mole) of crude p-chlorophenylhydrazine hydrochloride and 20 g. of sodium acetate in 400 ml. of dilute acetic acid was added. A yellow-brown precipitate of sticky crystals separated after a few mihutes. The reaction mixture was heated to 55° for thirty minutes and left overnight. The crystals were filtered off, washed with water and dried. The crude product, m.p. 87-95°, weighed 33 g. This represents a crude yield of 73%. The material was dissolved in chloroform, and Skelly B was added to the refluxing

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(30) Rydon and Long, Nature, 164, 575 (1949)

solution until a precipitate began forming. When the solution had cooled the precipitate was filtered off, washed with Skelly B and dried. This product weighed 2.4 g., and melted about 195°. It was not soluble in dilute sodium hydroxide so that it could not be the desired product. Further dilution of the mother liquor gave no more precipitate. Distillation of the mother liquor from the precipitate left a residue of a red oil, which would not crystallize. This oil was dissolved in ether, and a brown crystalline solid which did not dissolve was filtered off and washed with ether. This solid, insoluble in 1 M sodium hydroxide, weighed 2.2 g., m.p. 165-171°. The ether solution was extracted twice with dilute sodium hydroxide. Yellow crystals separated from the ether extract on standing a short tome. These were also base-insoluble, wt. 1.0 g., m.p. 193°. Ice was added to the aqueous layer and the cold solution carefully acidified with 10% hydrochloric acid. The product separated as an oil and was recovered by extraction with ether. The ether was dried over sodium sulfate and distilled. The crude oil obtained could not be crystallized from any of the following solvents: benzene, ethanol-water, chloroform-Skelly B, or dioxane-water. The crude oil was used for the next experiment.

The alkali-insoluble compound was not identified.

5-Chloroindole-3-acetic acid

A solution of 11.3 g. (0.050 mole) of crude succinaldehydic acid p-chlorophenylhydrazone, 30 ml. of concentrated sulfuric acid, and 270 ml. of commercial absolute ethanol was refluxed, under nitrogen, for five hours. The cooled solution was poured into 600 ml. of water and the oil which separated was extracted with five 200 ml. portions of ether. The combined ether extracts were dried over sodium sulfate containing a small amount of potassium carbonate to neutralize any acid in the ether. Distillation of the ether and alcohol left 10.1 g. of a dark red oil. This was transferred to a short path distillation apparatus, made from a large test tube, and distilled. The product came over rapidly at a bath temperature of 210-220° at a pressure of 0.2 mm. The distillate was a yellow oil weighing 5.8 g. The distillate was saponified by refluxing one hour with 60 ml. of 10% methanolic potassium hydroxide. One-hundred ml. of water was added and the methanol distilled off under reduced pressure. The aqueous solution was extracted with two 40 ml. portions of ether, which were discarded. Acidification of the aqueous solution gave a red oil which crystallized to sticky brown plates. This gave good crystals when washed with 10 ml. of chloro-

form. These melted 156-158°, wt. 2.5 g., yield 24%. The product was recrystallized from water (Norit A). The acid separated in beautiful white needles, m.p. 158-159 1/2°, wt. 2.0 g. The yield of pure 5-chloroindole-3-acetic acid was 20%.

Anal. Calcd. for $C_{10}H_8O_2NCl$: Neut. equiv., 209.6; N, 6.69

Found: Neut. equiv., 209.8, 212.0 (phenolphthalein indicator); N, 6.84, 6.72

Preparation of 7-Chlorindole-3-acetic Acid

o-Chlorophenylhydrazine hydrochloride

This compound was prepared by a modification of Hewitt's (31) procedure. Ninety grams (0.706 mole) of o-chloroaniline were dissolved in 1350 ml. of concentrated hydrochloric acid. The temperature of the reaction mixture was maintained between 0° and 5° while a solution of 52 g. (0.706 mole) of sodium nitrite in 360 ml. of water was added dropwise. When the diazotation was complete (negative starch-iodide test), a well-cooled solution of 320 g. (1.41 moles) of stannous chloride dihydrate in 300 ml. of concentrated hydrochloric acid was run in slowly. A white crystalline precipitate separated immediately. One hour after the addition of

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(31) Hewitt, J. Chem. Soc., 59, 209 (1891).

the stannous chloride solution was complete the product was filtered off, washed with two 100 ml. portions of concentrated hydrochloric acid and pressed free of excess solvent. The product was recrystallized from concentrated hydrochloric acid (3 liters). The hydrochloride was obtained in two crops: 71 g., m.p. 198° (dec.), and 31 g. containing some inorganic material which did not melt. Since the first crop was more than enough, the impure second crop was not purified further. The total yield was 81%.

Succinaldehydic acid o-chlorophenylhydrazone

This compound was prepared by the method of Stevens and Fox (2). Succinaldehydic acid was prepared in the usual way. A solution of 17.9 g. (0.10 mole) of o-chlorophenylhydrazine hydrochloride in 30 ml. of hot water was added to 0.074 mole (32) of succinaldehydic acid in 670 ml. of solution. An oil separated immediately. The solution was acidified to Congo red. The oil crystallized on standing. The crystals were filtered off, washed with water and dried. This gave 17 g. of crude product melting 100-150°. The yield of this crude

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(32) The concentration of the succinaldehydic acid was determined by treating an aliquot with an excess of p-nitrophenylhydrazine hydrochloride and weighing the hydrazone obtained.

product was 74%. Attempts to purify this compound were not successful. An attempt to recrystallize the crude material from benzene gave 5.2 g. of benzene insoluble α-ketoglutaric acid o-chlorophenylhydrazone melting 184° (dec.). A recrystallization from acetic acid raised the melting point to 185° (dec.) (33).

Anal. Calcd. for $C_{11}H_{11}O_4N_2Cl$: Neut. equiv., 135.3; Cl, 13.1

Found: Neut. equiv., 136 (potentiometric); Cl, 13.3, 12.7

Although Stevens and Fox describe the succinaldehydic acid o-chlorophenylhydrazone as a solid m.p. 180-185.5°, all attempts to obtain a crystalline product from the crude oil were fruitless. The crude oil was used for the next synthesis.

7-Chloroindole-3-acetic acid

A solution of 7.3 g. (0.032 mole) of crude succinaldehydic acid o-chlorophenylhydrazone, prepared above, 80 ml. of absolute ethanol and 8 ml. of concentrated sulfuric acid was refluxed, under nitrogen, for six hours. The cooled, dark-red solution was poured into 500 ml. of water and the ester extracted with three 150 ml. portions

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(33) A sample of this acid prepared by the Japp-Klingemann reaction melted 190 1/2° (dec.). See the following experiment.

of ether. The combined ether extracts were dried over sodium sulfate containing approximately 1% of sodium bicarbonate to neutralize any acid present. Distillation of the ether and alcohol left 7.5 g. of a dark red oil. This oil was transferred to a short path distilling apparatus and distilled at the lowest vacuum that could be obtained. Almost all of the product distilled 140-150° at 0.1 mm. The distillate, wt. 3.9 g., was saponified by refluxing one hour with 50 ml. of 10% methanolic potassium hydroxide. An equal volume of water was added and the solution distilled until the temperature of the vapor reached 99°. The cooled aqueous solution was acidified with 10% hydrochloric acid. The flask was cooled to about 0° and the crystals filtered off, washed with water, and dried. This gave 2.4 g. of crystals, m.p. 140-150°. The acid was washed with a small amount of chloroform; recrystallized from water and then from benzene. This gave 0.9 g. of pure 7-chloroindole-3-acetic acid, m.p. 166-167°. The yield was 13%.

Anal. Calcd. for $C_{10}H_8O_2NCl$: Neut. equiv., 209.6; N, 6.69

Found: Neut. equiv., 205 (potentiometric); N, 6.84, 6.39

Preparation of 7-Chloro-2-carboxyindole-3-acetic Acid

Ethyl α-ketoglutarate o-chlorophenylhydrazone

Six grams (0.026 mole) of ethyl α-acetoglutarate was dissolved in 25 ml. of ethanol. The solution was cooled to approximately -20° and 18.7 ml. of aqueous 20% sodium hydroxide were added. Immediately a solution of o-chlorophenyldiazonium chloride, prepared from 3.5 g. (0.027 mole) of o-chloroaniline, 1.9 g. (0.027 mole) of sodium nitrite, 7.6 ml. of concentrated hydrochloric acid and 15 ml. of water, was added. An orange oil separated. The mixture was shaken for a few minutes and allowed to warm up to room temperature. The solution was cooled again and acidified with 3 N hydrochloric acid. The solution was diluted with 100 ml. of water and the hydrazone, which did not crystallize, recovered by extraction with two small portions of ether. The combined ether extracts were dried over sodium sulfate and distilled. The crude product was used for the next synthesis without further purification.

Attempted cyclization of ethyl α-ketoglutarate o-chlorophenylhydrazone with alcoholic hydrogen chloride

The crude ethyl α-ketoglutarate o-chlorophenylhydrazone, prepared above, was dissolved in 25 ml. of

commericial absolute ethanol and refluxed 50 minutes while a rapid stream of dry hydrogen chloride was bubbled through the solution. No ammonium chloride was observed, cyclization, therefore, probably did not occur. The cooled solution was poured into 100 ml. of water and the oil extracted with two 50 ml. portions of ether. The combined ether extracts were distilled and the oily residue saponified by refluxing 30 minutes with a 10% ethanolic sodium hydroxide solution. The solution was cooled and the insoluble disodium salt filtered off and washed with ethanol. This salt, still wet with ethanol, was dissolved in 20 ml. of water, shaken with 0.5 g. of Norit A and filtered. Acidification with 3 N hydrochloric acid gave 1.1 g. of crystals, m.p. 188° (dec.). More product was recovered from the alkaline saponification solution by evaporation of the mother liquor and finally by acidification and ether extraction. The total yield of α-ketoglutaric acid o-chlorophenylhydrazone was 3.8 g. (0.014 mole) or 54% based on the ethyl α-acetoglutarate used. A pure sample, obtained by recrystallization from a water-ethanol mixture, melted 190 1/2° (dec.).

This compound was found to be identical with the product obtained in small amounts from the preparation of succinaldehydic acid o-chlorophenylhydrazone.

Cyclization of α-ketoglutaric acid o-chlorophenylhydrazone with ethanolic sulfuric acid

One gram (0.0037 mole) of α-ketoglutaric acid o-chlorophenylhydrazone was refluxed 2 1/2 hours with 15 ml. of commercial absolute ethanol and 3 ml. of concentrated sulfuric acid. The cooled reaction mixture was poured into 50 ml. of water and the milky solution extracted with three 25 ml. portions of ether. The combined ether extracts were washed with half-saturated sodium bicarbonate solution and with water. Distillation of the wet ether solution left a small amount of a dark oil, which was saponified by refluxing 30 minutes with 15% ethanolic sodium hydroxide. Very little solid separated during the refluxing, but on standing overnight the disodium salt separated in plates. These were filtered off and washed copiously with ethanol. The salt was dissolved in water, treated with 0.2 g. of Norit A and filtered. Acidification gave 0.3 g. (0.0012 mole), 31%, of powdery crystals, m.p. 255° (dec.). The acid was recrystallized from a water-ethanol mixture which raised the melting point to 256° (dec.). A second recrystallization from a water-acetic acid solution did not increase the melting point. Neither solvent pair could be considered satisfactory for recrystallization of the compound as most of the mother liquor had to be distilled

off to recover the acid in both cases.

Anal. Calcd. for $C_{11}H_8O_4NCl$: Neut. equiv., 126.8; N, 5.54

Found: Neut. equiv., 125 (potentiometric); N, 5.56, 5.72

Preparation of 5,7-Dichloroindole-3-acetic Acid

Succinaldehydic acid 2,4-dichlorophenylhydrazone

To a solution of succinaldehydic acid, prepared from 0.2 mole of glutamic acid in the usual manner, was added a hot solution of 30.5 g. (0.12 mole) of 2,4-dichlorophenylhydrazine hydrochloride in 400 ml. of 25% acetic acid. An oil separated immediately. The solution was stirred for thirty minutes and allowed to cool. The sticky, brown tar was extracted with three 150 ml. portions of ether. The combined ether extracts were dried over sodium sulfate. Distillation of the ether and acetic acid left 23.5 g. of a mixture of oil and crystalline solid. The crude product was warmed with 200 ml. of benzene and an insoluble, yellow, crystalline solid filtered off and washed with benzene. This gave 2.9 g. (0.0095 mole), 5% of the glutamic acid, as crude α-ketoglutaric acid 2,4-dichlorophenylhydrazone melting 205-206° (dec.) The product was purified by recrystallization from a water-acetic acid solution and from a water-

ethanol mixture. The pure acid melted 215-216° (dec.). A sample of this acid recovered from an attempted cyclization melted 223-224° (dec.). (See the following experiment.)

Anal. Calcd. for $C_{11}H_{10}O_4N_2Cl_2$: Neut. equiv., 152.6; Cl, 23.2

Found: Neut. equiv., 154, 155 (phenolphthalein); Cl, 22.6, 23.0

Only a few crystals of the succinaldehydic acid 2,4-dichlorophenylhydrazone separated from the benzene solution on cooling, and dilution of the benzene solution with petroleum ether, b.p. 60-70°, gave no more product. Distillation of the benzene-petroleum ether left an orange oil, which could not be crystallized from a water-ethanol mixture. The oil was recovered by extraction of the solution with ether. Distillation of the dried (sodium sulfate) ether and alcohol solution left 14 g. of an orange oil (34), which was used for the cyclization reaction without any further attempts at purification. The yield based on the crude oil and the glutamic acid used was 26%.

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(34) Succinaldehydic acid 2,4-dichlorophenylhydrazone melts 181-182° (dec.). See Stevens and Fox, J. Am. Chem. Soc., 70, 2263 (1948).

5,7-Dichloroindole-3-acetic acid

A solution of 14 g. (0.53 mole) of the crude succinaldehydic acid 2,4-dichlorophenylhydrazone, prepared above, 360 ml. of absolute ethanol, and 40 ml. of concentrated sulfuric acid were refluxed in a nitrogen atmosphere for six hours. The cooled solution was poured into 1 liter of water, and the oil which separated was extracted with three 150 ml. portions of ether. The combined ether extracts were washed with a half-saturated sodium bicarbonate solution and with water, and then dried over sodium sulfate. Distillation of the ether and ethanol left 11.5 g. of black oil which was purified by vacuum distillation from a molecular still made from a test tube. The product distilled 150-160° at 0.1 to 0.05 mm. The distillate, wt. 8.5 g., was a mixture of crystals and oil. The crude ester was saponified by refluxing 30 minutes with 40 ml. of 10% ethanolic sodium hydroxide solution. The solution was diluted with 100 ml. of water and distilled until the temperature of the vapor reached 98°. The cooled solution was extracted once with ether, which was discarded. The aqueous solution was triturated with0.2 g. of Norit A and filtered. Acidification of the filtrate with 10% hydrochloric acid gave a brown tar. This tar was recovered by decantation, dried, and treated with 10 ml. of hot

chloroform which caused the product to crystallize. This gave 1.7 g. of product, m.p. 140-152° (dec.). Concentration of the chloroform gave an additional 1.0 g. of product, m.p. 173-180° (dec.). The total yield of crude product was 2.7 g. (0.011 mole) or 21%. Both crops were recrystallized separately from a water-ethanol solution, and both products melted 182-183° (dec.). The compounds were treated with hot benzene and a small amount of the benzene-insoluble α-ketoglutaric acid 2,4-dichlorophenylhydrazone was filtered off. This product had a melting point of 215° (dec.), and a mixed melting point with an authentic sample of α-ketoglutaric acid 2,4-dichlorophenylhydrazone was not depressed. Evaporation of most of the benzene gave products melting 189-190° (dec.) and 190-193° (dec.). Extraction of the product with boiling water gave a product melting 194-197° (dec.)., and a smaller amount which crystallized out of the water on cooling also melted 194-197° (dec.). The yield of pure product was 1.2 g. (0.0049 mole) or 9%.

Anal. Calcd. for $C_{10}H_7O_2NCl_2$; Neut. equiv., 244.1, N, 6.13

Found: Neut. equiv., 249; (potentiometric); N, 5.74, 5.65

Attempted Cyclization of α-Ketoglutaric Acid 2,4-Dichlorophenylhydrazone with Ethanolic Sulfuric Acid

A solution of 0.7 g. (0.0023 mole of α-ketoglutaric acid 2,4-dichlorophenylhydrazone, 2 ml. of concentrated sulfuric acid and 18 ml. of absolute ethanol was refluxed, under nitrogen, for 8 1/2 hours. The cooled solution was poured into 100 ml. of water and the oil which separated extracted with one 150 ml. and two 25 ml. portions of ether. The combined ether extracts were washed with a dilute sodium bicarbonate solution and with water. Distillation of the combined, wet ether extracts left a low melting crystalline solid. This was saponified by refluxing 40 minutes with 20 ml. of a 10% ethanolic sodium hydroxide solution. A small amount of crystals separated during the refluxing. The mixture was left standing overnight, and the small amount of crystals filtered off and washed with absolute ethanol. It was observed that dilution of the filtrate with absolute ethanol caused more crystals to form. The mother liquor was diluted to 50 ml. with absolute ethanol and a second crop recovered as before. The two crops were combined, dissolved in 10 ml. of water, warmed with 0.05 g. of Norit A and filtered. Acidification of the filtrate with 10% hydrochloric acid gave white powdery crystals. The

suspension was warmed 20 minutes to digest the crystals, cooled and filtered. The crystals were washed with water and air dried. This gave 0.6 g. (0.002 mole), 86%, of recovered α-ketoglutaric acid 2,4-dichlorophenylhydrazone melting 223° (dec.). The compound separated in fine needles from a water-ethanol mixture, m.p. 223-224° (dec.). The recrystallized product weighed 0.5 g.

Anal. Calcd. for $C_{11}H_{10}O_4N_2Cl_2$: N, 9.17

Found: N, 9.16, 9.15

A mixed melting point with the starting material, m.p. 215-216° (dec.), was 219-220° (dec.).

Preparation of 5-Methylindole-3-acetic Acid and 5-Methyl-2-carboxyindole-3-acetic Acid

Succinaldehydic acid p-tolylhydrazone

To a solution of succinaldehydic acid prepared from 29.4 g. (0.2 mole) of glutamic acid in the usual manner was added a filtered solution of 23.8 g. (0.15 mole) of p-tolylhydrazone hydrochloride in 40 ml. of 3 N hydrochloric acid and 400 ml. of water. The product separated as an oil which did not crystallize on standing overnight. The oil was extracted with two 200 ml. portions of ether. The combined ether extracts were dried over sodium sulfate and distilled. This gave 19.8 g. (0.095 mole) of a dark oil. This was a yield of 48% based on the 0.2

mole of glutamic acid used. The oil was soluble in benzene and insoluble in petroleum ether. No crystals could be obtained from these solvents nor by dissolving the product in dilute sodium hydroxide and precipitating the free acid by the slow addition of hydrochloric acid. The crude product was used in the next experiment.

5-Methylindole-3-acetic acid and 5-methyl-2-carboxyindole-3-acetic acid

A solution of 19.8 g. (0.095 mole) of crude succinaldehydic acid p-tolylhydrazone, prepared above, 360 ml. absolute ethanol and 40 ml. of concentrated sulfuric acid was refluxed, under nitrogen, for four and one-half hours. After the dark solution had cooled it was poured into 1 liter of ice water, and the oil which separated was extracted with three 200 ml. portions of ether. The combined ether extracts were washed once with half-saturated sodium bicarbonate, once with water, and dried over sodium sulfate. Distillation of the ether left 18.8 g. of an oil. This crude ester was transferred to a crude molecular still, made from a large test tube, and distilled at 0.2 mm. The oily distillate, wt. 8.2 g., was washed from the still with 40 ml. of absolute ethanol; 35 ml. of 10% ethanolic sodium hydroxide solution was added and the esters saponified

by refluxing 40 minutes. After the solution had cooled the insoluble disodium salt of 5-methyl-2-carboxyindole-3-acetic acid was filtered off and washed copiously with absolute ethanol. This salt was dissolved in water and the free acid precipitated by acidification with 3 N hydrochloric acid. The acid was filtered off, washed with water and dried. This gave 1.5 g. of the 5-methyl-2-carboxyindole-3-acetic acid, m.p. 240-241° (dec.). A recrystallization from water containing a small amount of ethanol did not raise the melting point.

Anal. Calcd. for $C_{12}H_{11}O_4N$: Neut. equiv., 116.6; N, 6.01

Found: Neut. equiv., 116.6 (potentiometric); N, 6.39, 5.83

The alkaline solution from which the insoluble salt was filtered was diluted with 150 ml. of water and distilled until all the ethanol had distilled over. The solution was shaken with 1 g. of Norit A and filtered. Acidification of the filtrate gave almost white crystals which were filtered off, washed with water and dried. This gave 5.5 g. (0.029 mole), 31%, of acid m.p. 135° (dec.). One recrystallization from water (Norit A) gave pure 5-methylindole-3-acetic acid, m.p. 151-152° (35).

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(35) Kogl and Kostermans, Z. Physiol. Chem., 235, 201-16 (1935).

An attempted cyclization of the crude p-tolylhydrazone with ethanolic hydrogen chloride gave a precipitate of ammonium chloride, indicating that cyclization had occurred. However, an attempt to isolate a pure product by saponification of the undistilled cyclization product was not successful.

Preparation of 4-Chloroindole-3-acetic Acid

The intermediates through 4-chloroindole were prepared by the method of Uhle (36).

2-Nitro-6-chlorophenylpyruvic acid

One mole of sodium ethoxide was prepared by dissolving 23 g. (1 mole) of sodium in 350 ml. of absolute ethanol. The ethoxide solution was cooled and 146 g. (1 mole) of ethyloxalate was added. This was followed by 171.6 g. (1 mole) of technical grade 2-nitro-6-chlorotoluene. The deep-red solution was warmed on the steam bath with frequent shaking until all the solid had dissolved. The solution was then maintained at reflux temperature for forty minutes and allowed to cool. The solution was diluted with 200 ml. of water, and steam distilled until no more starting material came over

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(36) Uhle, J. Am. Chem. Soc., 71, 761 (1949).

(5-6 liters). This gave 84.5 g., 49%, of recovered 2-nitro-6-chlorotoluene. The distillation residue was clarified by shaking with a mixture of 10 g. of diatomaceous earth and 6 g. of Norit A and filtering. A large amount of black tar (36 g.) was discarded. The cooled filtrate was acidified to pH 2 by the slow addition of 10% hydrochloric acid. The product separated as a dark oil, but crystallized when the flask was shaken vigorously. The crystals were filtered off, washed with water and dried. This gave 69 g., 28%, of crude product melting 100-106°. After one recrystallization from benzene the product melted 106-108°. This crude acid was used for the next synthesis without further purification.

4-Chloroindole-2-carboxylic acid

To a solution of 60.3 g. (0.244 mole) of crude 2-nitro-6-chlorophenylpyruvic acid, prepared above, in 250 ml. of 4 N ammonium hydroxide was added a suspension of ferrous hydroxide, prepared from 410 g. (1.47 moles) of ferrous sulfate heptahydrate in 180 ml. of NH_4OH and 1,500 ml. of water. The red color of the pyruvic acid derivative disappeared immediately. The suspension was boiled for five minutes and filtered. The ferric hydroxide was washed with dilute ammonium hydroxide until the filtrate gave no precipitate when acidified. The filtrates were acidified to pH 2, cooled, and the white, finely divided precipitate filtered off and washed with

water. This gave 44.0 g. of fairly pure product, melting 256°. The yield was 92%. This product was pure enough for use in the next synthesis so was not recrystallized.

4-Chloroindole

Twenty-two and two-tenths grams (0.225 mole) of cuprous chloride was dissolved in 200 ml. of freshly distilled technical grade quinoline. The solution was heated to boiling and approximately 5 ml. of quinoline was distilled to remove all moisture. The solution was cooled (solidified) and 44 g. (0.225 mole) of 4-chloroindole-2-carboxylic acid, prepared above, was added. The system was swept out with nitrogen and connected to a trap. The flask was heated slowly with frequent shaking until all the material had dissolved. The decarboxylation began when the temperature of the bath reached 225°. The bath temperature was maintained at 235-240° for five hours. The solution was cooled to about 150° and poured with vigorous stirring into 1 liter of ice and dilute hydrochloric acid. The large amount of precipitate which formed did not dissolve when ether was added to the aqueous suspension. The solid was filtered off, washed several times with ether and discarded. The ether layer was separated and the aqueous layer extracted with three 100 ml. portions of ether. The combined ether extracts were washed once with dilute hy-

drochloric acid and once with half-saturated sodium bicarbonate solution. The ether solution was dried over sodium sulfate and distilled. The black oily residue was partially purified by distillation through a six-inch Vigreux column. The main portion distilled 121-125° at 1.5 mm. The yield of this fairly pure product was 22 g., 62%. The index of refraction of the product was 1.6283 at 20°. Uhle gave a value of 1.6254. The products were recombined and purified further by vacuum fractionation through a thirteen plate column. The fractions were collected as follows:

No.	B.P.°	Press.	Wt., g.	N_D^{20}	d_{20}^{20}
I	87-100	25mm.	2.3		
II	100-102	"	0.7	1.5461	
III	111-150	25-13mm.	3.0	1.6029	
IV	150-150	13mm.	13.7	1.6286	1.259
V	150-151.5	"	4.1	1.6284	

The yield of pure product (fractions IV and V) was 17.8 g., (0.115 mole), 51%. Since the physical constants of the pure product were appreciably different from those given by Uhle the product (fraction IV) was analyzed.

Anal. Calcd. for C_8H_6NCl: M_{rD}, 42.4; Cl, 23.4

Found: Mr_D, 42.7; Cl, 23.3, 23.3

4-Chlorogramine

This preparation was patterned after the preparation of 4-cyanogramine described by Uhle (36).

A solution of 8 g. (0.053 mole) of 4-chloroindole, 4.3 g. (0.052 mole) of 36% aqueous formaldehyde, 9.3 g. (0.052 mole) of 25% aqueous dimethylamine, and 26 ml. of acetic acid was left standing at room temperature overnight. The acetic acid was distilled under diminished pressure. A small amount of tar separated when the solution was diluted with 75 ml. of water. The solution was decanted from the tar, shaken with 1 g. of Norit A and filtered. Alkalization of the filtrate with 1 M sodium hydroxide gave a white curdy precipitate which changed to a beautiful white crystalline solid on standing in the ice box. These crystals were filtered off, washed with water and dried. This gave 8.4 g. (0.040 mole) of product, m.p. 144-147°. The yield was 78% based on the formaldehyde used. A small amount was purified by recrystallization from acetone to obtain an analytical sample. The product melted 147-148 1/2° after the first and second recrystallizations. The melting point seems to be slightly dependent on the rate of heating. If the heating is fairly rapid the melting point is increased to 150-151°.

Anal. Calcd. for $C_{11}H_{13}N_2Cl$: Cl, 17.0

Found: Cl, 17.1, 17.1

Attempted preparation of 4-chloroindole-3-acetic acid from 4-chlorogramine

This synthesis was patterned after the synthesis described by Snyder (37) for the preparation of indol-3-acetic acid from gramine.

A solution of 4 g. (0.0192 mole) of 4-chlorogramine, 0.94 g. (0.0192 mole) of sodium cyanide, 37 ml. of 95% ethanol and 9.5 ml. of water was refluxed for ninety hours. The solution was worked up in the manner described by Snyder. No 4-chloroindole-3-acetamide could be obtained and only a small yield, 0.17 g. of crude acid, m.p. 141-160°, could be obtained. The principal product of the reaction was an unidentified solid, insoluble in acids or bases, which gave on alkaline hydrolysis a base soluble substance melting over 360°. This product was not characterized further.

Preparation of 4-chloroindole-3-acetic acid from 4-chloroindole

This synthesis was patterned after the method used by Majima and Hoshino (38) for the preparation of indole-3-acetic acid.

A solution of ethylmagnesium iodide was prepared in

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(37) Snyder, J. Am. Chem. Soc., 70, 3771 (1948).
(38) Majima and Hoshino, Ber., 58, 2042 (1925).

the conventional manner. To a mechanically stirred solution of 6.6 g. (0.0435 mole) of 4-chloroindole in 20 ml. of sodium-dried ether was added an equimolar quantity (39) of ethylmagnesium iodide in 48.4 ml. of ether. The temperature of the reaction flask was maintained at 0° during the addition of the Grignard solution by cooling in an ice bath. The solution became a light green color and became fairly viscous. Color Test I (40) was observed to be negative five minutes after the addition of the Grignard. After standing at room temperature for an hour the solution was cooled in an ice bath and a solution of 3.3 g. (0.0435 mole) of chloroacetonitrile in 15 ml. of ether was added dropwise over a period of 10 minutes. The solution, now in two phases, was stirred at 0° for 30 minutes and then refluxed for two hours. The color of the lower phase changed from a light green to a red-brown color during this time. A solution of 2.6 g. (0.0435 mole) of acetic acid in 15 ml. of water was added; the solution stirred a few minutes and left standing four hours. During this time the whole solution became a dark red color. The ether layer was separated and the aqueous layer extracted repeatedly with benzene. The combined ether and benzene extracts were

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(39) The titer of the Grignard was determined by titration.

(40) Gilman and Schulze, J. Am. Chem. Soc., 47, 2002 (1925).

dried over sodium sulfate and distilled. The black oily residue was purified by vacuum distillation. The products were collected as follows:

No.	B.P.°	Press.	Wt., g.	Remarks
I	100-155	0.2mm.	3.5	Distillate a black oil
II	155-176	"	0.6	" " " "
III		"	1.0	Recovered from condenser

Since it was apparent that the fractions were all mixtures no attempt was made to characterize the nitrile, but instead the fractions were mixed and hydrolyzed by refluxing 4 hours with a solution of 10 ml. of methanol and 20 ml. of aqueous 20% potassium hydroxide. Almost all of the oily second phase disappeared during the hydrolysis. The cooled alkaline solution was filtered through a layer of diatomaceous earth. The filtrate was extracted with three 20 ml. portions of ether to remove the methanol and non-acid components. After treatment with a small amount of Norit A, ice was added, and the solution acidified by the slow addition of concentrated hydrochloric acid. The 4-chloroindole-3-acetic acid separated in light brown crystals. These were filtered off, washed with water and dried. The product melted 185-187° (dec.) and weighed 1.7 g. (0.00811 mole). The yield was 19%. After one recrystallization from water

the product melted 186-188° (dec.). The acid is very soluble in ethanol, almost completely insoluble in benzene or chororform, and very difficultly soluble in water.

Anal. Calcd. for $C_{10}H_8NO_2Cl$: Neut. equiv., 209.62; N, 6.69

Found: Neut. equiv., 210.5 (potentiometric); N, 6.85, 6.78

Attempted Preparation of 6-Chloroindole by Tyson's Method (41)

N-Formo-4-chlorotoluidid

This compound was prepared by the procedure described by Tyson for the preparation of N-formotoluidid. One hundred grams (0.706 mole) of 2-amino-4-chlorotoluene and 34.6 g. (0.741 mole) of 99% formic acid were placed in a 250 ml. flask fitted with an air condenser and heated on the steam bath for four hours and left standing overnight. The solid product was crushed with 100 ml. of water, the crystals filtered off, and washed with very dilute hydrochloric acid and water. This gave 112.5 g. of product, m.p. 128-131°. One recrystallization from benzene gave 97.3 g. of pure product, m.p. 130 1/2-

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(41) Tyson, Org. Syn., 23, 42 (1943).

131 1/2°. The yield was 81%.

Anal. Calcd. for C_8H_8ONCl: Cl, 20.9

Found: Cl, 21.0 and 20.8

Attempted preparation of 4-chloroindole

The preparation of this compound was attempted using the procedure worked out by Tyson for the preparation of indole. In a 1-liter 3-neck flask fitted with a reflux condenser and inlet for dry nitrogen were placed 240 ml. of t-butyl alcohol and 11.6 g. (0.297 mole) of potassium. The flask was warmed until the potassium had dissolved, and 33.9 g. (0.20 mole) of N-formo-4-chlorotoluidid was added. The t-butyl alcohol was distilled off and the residue heated to 350-360° in a Wood's metal bath for twenty minutes. Gas was evolved and a small quantity of high boiling liquid distilled. After the flask had cooled in a stream of nitrogen, 100 ml. of water was added and the mixture was subjected to steam distillation. Only traces of material other than t-butyl alcohol distilled, and the product separated in the still pot as a tarry solid. This black tar was extracted with ether. Distillation of the dried ether extracts gave 9.5 g. of a black tar. An attempted purification by vacuum distillation gave 1 g. of a red viscous oil, b.p. 170-186° at 0.4mm. This

could not be crystallized from a benzene-Skelly B mixture nor from methanol. An attempt to isolate some product as the picrate gave a few milligrams of crystals, m.p. 160° after three recrystallizations from methanol. Thes could not be the picrate of 6-chloroindole which melts 143-144° (30). This procedure for the preparation of halogenated indoles appears unsatisfactory. The experiment was not carried further.

Preparation of 6-Chloroindole-3-acetic Acid

2-Nitro-4-chlorophenylpyruvic acid

To a solution of sodium ethoxide, prepared by dissolving 13.4 g. (0.58 mole) of sodium in 204 ml. of absolute ethanol, 85 g. (0.58 mole) of ethyl oxalate and 100 g. (0.58 mole) of 2-nitro-4-chlorotoluene was added. The solution became dark red and the ethanol refluxed gently for five minutes from the heat of reaction. The solution, protected from the atmosphere by an oil trap, was refluxed for forty-five minutes and left standing one hour. The solution was diluted with 300 ml. of water and steam distilled until no more starting material came over. This gave 27 g., 27%, of product, m.p. 38-41°. A mixed melting point with an authentic sample of 2-nitro-4-chlorotoluene was 38-41°.

The distillation residue was made slightly alkaline

by the addition of 10 ml. of N sodium hydroxide in an attempt to dissolve the solid present. The solution was decanted through a thick layer of diatomaceous earth to remove a large amount of tar. Acidification of the filtrate with 10% hydrochloric acid gave a dark oil which crystallized on standing overnight. The very crude product was recrystallized from benzene and from water with a large amount of decolorizing charcoal employed with each solvent. This gave 25 g. of product melting 129-134° and 4 g. of pure product melting 138-140°. The total yield was 29 g. (0.124 mole) or 21%.

Anal. Calcd. for $C_9H_6NO_5Cl$: Neut. equiv., 243.5; Cl, 14.6

Found: Neut. equiv. 245, 243 (phenolthalein); Cl, 14.5, 14.6

6-Chloroindole-2-carboxylic acid

A suspension of ferrous hydroxide, prepared from 173 g. (0.618 mole) of ferrous sulfate heptahydrate in 65 ml. of ammonium hydroxide and 560 ml. of water, was added to 25 g. (0.103 mole) of crude 2-nitro-4-chlorophenylpyruvic acid in 75 ml. of water and 25 ml. of ammonium hydroxide. The suspension was boiled for five minutes and the ferric hydroxide filtered off and washed with four 100 ml. portions of dilute ammonium hydroxide.

Acidification of the filtrate with 10% hydrochloric acid gave a white finely divided precipitate. This was filtered off, washed generously with water and pressed free of excess solvent. This gave 11.4 g. (0.0584 mole), 56%, of product melting 240-241° (dec.). After one recrystallization from a water-ethanol mixture the acid melted 241-242° (dec.). A second recrystallization from the same solvent raised the melting point to 242-244° (dec.).

Anal. Calcd. for $C_9H_6O_2NCl$: Neut. equiv., 195.5; Cl, 18.1

Found: Neut. equiv., 191.5, 193 (phenolphthalein); Cl, 17.8, 17.8

6-Chloroindole

Five grams of cuprous chloride was dissolved in 60 ml. of technical grade quinoline and approximately one ml. of quinoline was distilled to remove all traces of water. After the solution had cooled to about 150°, 8.8 g. (0.045 mole) of 6-chloroindole-2-carboxylic acid was added. The flask was connected through a reflux condenser to a bubble counter and heated slowly in a Wood's metal bath until decarboxylation began. The evolution of CO_2 commenced at about 200°, but the bath temperature was maintained at 240° until the evolution of gas had stopped (3 hours). The solution was cooled and triturated with ether. Water was added and the mix-

ture shaken vigorously. A large amount of solid was filtered off and washed alternately with dilute hydrochloric acid and ether. The ether-water mixture was made acid to Congo red with concentrated hydrochloric acid and the ether layer separated. The ether solution was washed twice with water and once with half-saturated sodium bicarbonate solution. After the ether solution had been dried over sodium sulfate and distilled there remained 7.2 g. of a black oil which solidified on cooling. The crude product was purified by vacuum distillation through a short Vigreux column. After one gram of forerun the product distilled at 90° at 0.3 mm. This gave 5.0 g. (0.033 mole), 74%, of crystals melting 83-86°. The melting point of pure 6-chloroindole is 89-90° (30), but this product was sufficiently pure for the next synthesis.

6-Chloroindole-3-acetic acid

This synthesis was patterned after the work of Majima and Hoshino (38). A solution of ethylmagnesium iodide was prepared in the conventional manner. To a solution of 5 g. (0.033 mole) of 6-chloroindole, prepared above, in 25 ml. of sodium-dried ether was added 35 ml. (0.033 mole) of the Grignard solution. The addition was carried out with cooling in an ice bath and the

solution was stirred at this temperature for one hour. A solution of 2.8 g. (0.037 mole) of chloroacetonitrile in 25 ml. of ether was added dropwise at this same temperature. The solution was stirred at 0° for thirty minutes and then at reflux temperature for 4 hours. The solution was cooled in an ice bath and a cold solution of 3 ml. of acetic acid in 50 ml. of water was added. Fifty ml. of benzene were added and the reaction mixture left standing overnight. The ether-benzene layer was separated and the water layer extracted with two 50 ml. portions of benzene. An estimated 1 g. of material which did not dissolve in either layer was discarded. The combined benzene-ether extracts were dried over sodium sulfate and distilled. The black viscous residue was transferred to a 50 ml. flask and vacuum distilled through a short vacuum-jacketed Claisen still head. Two grams of black oil distilled from 160-180° at 0.2 mm. The distillate was saponified by refluxing 4 hours with 10 ml. of methanol and 20 ml. of 20% aqueous potassium hydroxide. The basic solution was triturated with 0.1 g. of Norit A and filtered through a layer of diatomaceous earth. The filtrate was extracted with two 40 ml. portions of ether which were discarded. Acidification of the aqueous layer with concentrated hydrochloric acid gave a crystalline (plates) product. The crystals were

filtered off, washed generously with water and dried. This gave 1.8 g. (0.0086 mole) of acid, m.p. 185-186° (dec.). The yield was 26% based on the 6-chloroindole used. One recrystallization from 300 ml. of water (Norit A) raised the melting point to 187-188° (dec.).

Anal. Calcd. for $C_{10}H_8O_2NCl$: N, 6.69

Found: N, 6.54, 6.59

Preparation of 4-Chloroindole-3-acetic Acid and 6-Chloroindole-3-acetic Acid from Succinaldehydic Acid m-Chlorophenylhydrazone

Succinaldehydic acid m-chlorophenylhydrazone

To a solution of 29.4 g. (0.2 mole) of glutamic acid in 400 ml. of 0.5 N sodium hydroxide was added 0.2 mole of sodium hypochlorite in 208 ml. of solution. The solution was warmed to 50° on the steam bath (starch-iodide test negative) and acidified by the addition of 70 ml. of 3 N hydrochloric acid. Carbon dioxide was evolved during the acidification. The solution was maintained at 50° until the solution gave a negative starch-iodide test (10 minutes). A solution of 17.1 g. (0.12 mole) of m-chlorophenylhydrazine dissolved in 50 ml. of 25% acetic acid was added. A thick oil separated immediately. Fifty more ml. of 3 N HCl was added and

the stirring continued for two hours while the reaction mixture cooled to room temperature. The oil was extracted with three 200 ml. portions of ether. The combined ether extracts were dried over sodium sulfate and distilled. Most of the acetic acid was also distilled off on the steam bath by reducing the pressure with an aspirator. Extraction of the oil with 200 ml. of benzene left 2.3 g. (0.0085 mole), 4% of the glutamic acid, of crude α-ketoglutaric acid m-chlorophenylhydrazone melting 174° (dec.). Two recrystallizations from a water-ethanol mixture gave 2.0 g. of the pure phenylhydrazone, m.p. 182° (dec.).

Anal. Calcd. for $C_{11}H_{11}O_4N_2Cl$: Neut. equiv., 135.3; N, 10.30

Found: Neut. equiv., 135.2 (potentiometric); N, 10.44, 10.28

Concentration of the benzene extract to 25 ml. gave 13.1 g. of crystals, m.p. 105°. Further concentration gave 11.2 g. of an oil which did not crystallize. An attempt to recrystallize the crystalline product from benzene gave 0.5 g. of crude α-ketoglutaric acid m-chlorophenylhydrazone, m.p. 173° (dec.), and 9.4 g. of the desired product, m.p. 101-102°. Two recrystallizations from a water-ethanol mixture did not raise the melting point. All attempts to crystallize the oil fraction

were unsuccessful. The yield, including the crude oil which was successfully used in a cyclization reaction, was 24.4 g. (0.108 mole) or 54% based on the glutamic acid used.

Anal. Calcd. for $C_{10}H_{11}O_2N_2Cl$: Neut. equiv., 226.6; N, 12.34

Found: Neut. equiv., 226.2 (potentiometric); N, 12.41, 12.50

Cyclization of succinaldehydic acid m-chlorophenylhydrazone

A solution of 7.2 g. (0.0317 mole) of the crystalline succinaldehydic acid m-chlorophenylhydrazone, prepared above, 200 ml. of absolute ethanol and 21 ml. of concentrated sulfuric acid was refluxed in a nitrogen atmosphere for six hours. The cooled solution was poured into 1 liter of water and the oil which separated was extracted with three 150 ml. portions of ether. The combined ether extracts were washed with half-saturated sodium bicarbonate solution and dried over sodium sulfate. Distillation of the ether and alcohol left a dark oil, which was transferred to a crude molecular still and vacuum distilled. The ester distilled 140-150° at 0.05 mm. The distillate, 3.5 g. of a yellow oil, was saponified by refluxing 40 minutes with 25 ml. of a

10% ethanolic sodium hydroxide solution. The alkaline solution was diluted with 50 ml. of water and distilled until the temperature of the vapor reached 98°. The cooled solution was extracted with a small amount of ether which was discarded. The aqueous solution was shaken with 0.2 g. of Norit A and filtered. Acidification of the filtrate gave an oil which crystallized on standing several hours. The sticky crystals were filtered off and washed with water. The product weighed 2 g. but was too sticky for a melting point determination. Extraction of the product with 5 ml. of chloroform left 1.6 g. (0.0076 mole) of crystals melting 151-160°. This represents a yield of 24%. The product was refluxed with a small amount of benzene. A fraction which did not dissolve was filtered off, wt. 0.4 g., and recrystallized from water. The product separated in plates, m.p. 185-186° (dec.). A mixed melting point of this compound with an authentic sample of 6-chloroindole-3-acetic acid showed no depression. Concentration of the benzene gave crystals (plates) of an eutectic mixture of the 4-chloro- and 6-chloroindole-3-acetic acids, m.p. 154-159° (dec.). After one recrystallization from water and one from benzene the eutectic mixture melted 158-159° (dec.). The melting point of this mixture was not depressed by either the 4- or 6-chloroindole-

3-acetic acids. The identity of the eutectic mixture was established by Dr. A. I. Snow by comparison of the powder X-ray diffraction patterns of the mixture and of the two pure components. The eutectic mixture contained more 6-chloroindole-3-acetic acid than 4-chloroindole-3-acetic acid.

Anal. Calcd. for $C_{10}H_8O_2NCl$: Neut. equiv., 209.6; N, 6.69

Found: Neut. equiv., 211 (potentiometric); N, 6.50, 6.78

Cyclization of 10 g. of the oil fraction gave 1.1 g. of crude crystals melting 144-150°. This product was worked up as above, and the mother liquors of the two cyclizations were combined and worked up in the conventional manner. The total combined yield of pure eutectic mixture from the two cyclizations was 2.6 g., or 16%.

Preparation of 7-Methyl-2-carboxyindole-3-acetic Acid

Ethyl α-ketoglutarate o-tolylhydrazone

A solution of o-tolyldiazonium chloride, prepared from 5.4 g. (0.05 mole) of o-toluidine, 12.5 ml. of concentrated hydrochloric acid, 22 ml. of water and 3.5 g. (0.051 mole) of sodium nitrite, was added to a solution of 10 g. (0.044 mole) of ethyl α-acetoglutarate in 42 ml. of ethanol and 31 ml. of 20% aqueous sodium hydroxide at

-20°. An orange oil separated immediately. The suspension was left in an ice-salt bath for ten minutes and acidified to Congo red with 10% hydrochloric acid. The red oil was extracted with 60 ml.of ether. The ether extract was washed with 50 ml. of saturated sodium bicarbonate solution, dried over sodium sulfate and distilled. This left 7.5 g. of a dark red oil which was used in the cyclization reaction without any attempts at purification or crystallization.

Methyl-2-carboxyindole-3-acetic acid

The crude ester, prepared above, was refluxed 2 1/2 hours, in a nitrogen atmosphere, with a solution of 7 ml. of concentrated sulfuric acid and 63 ml. of absolute ethanol. The cooled solution was poured into 300 ml. of ice water and the oily ester extracted with two 50 ml. portions of ether. The combined ether extracts were washed with saturated sodium bicarbonate solution and distilled. The dark oily residue was saponified by refluxing 40 minutes with 40 ml. of a 10% ethanolic sodium hydroxide solution. The disodium salt began separating immediately. The solution was diluted to 80 ml. with absolute ethanol and left standing overnight. The insoluble, crystalline disodium salt was filtered off and washed copiously with absolute ethanol. The

salt was dissolved in water, triturated with a small amount of Norit A and filtered through a layer of diatomaceous earth. Acidification of the filtrate with 10% hydrochloric acid gave 1.4 g. (0.0060 mole), 13.8%, of crude acid melting 204-207° (dec.). The product was purified by an extraction with chloroform followed by a recrystallization from a water-ethanol mixture. This gave 0.7 g. of acid melting 227-228° (dec.). After further purification by precipitation of the disodium salt from ethanol and reprecipitation of the acid with 10% hydrochloric acid the acid melted 228-229° (dec.). Recrystallization from water did not raise the melting point. The yield of the pure 7-methyl-2-carboxyindole-3-acetic acid was 0.59 g. (0.0025 mole) or 6%. The compound gives a reddish-violet Ehrlich test which changes to a deep violet on standing.

Anal. Calcd. for $C_{12}H_{11}O_4N$: Neut. equiv., 116.5; N, 6.01

Found: Neut. equiv., 113.2 (potentiometric); N, 6.10, 5.95

Preparation of 5-Bromo-2-carboxyindole-3-acetic Acid

Ethyl α-ketoglutarate p-bromophenylhydrazone

Ten grams (0.0435 mole) of ethyl α-acetoglutarate

were dissolved in 42 ml. of 95% ethanol. The solution was cooled to -20° and 31 ml. of cold aqueous 20% NaOH was added. Immediately a solution of p-bromophenyldiazonium chloride, prepared from 8.6 g. (0.05 mole) of p-bromoaniline, 3.5 g. (0.05 mole) of sodium nitrite in 35 ml. of water, and 12.5 ml. of concentrated hydrochloric acid was added. A yellow oil separated immediately. The suspension was stirred for five minutes and acidified by the addition of 25 ml. of concentrated hydrochloric acid. The oil changed to a bright red color but showed no tendency to crystallize. The oil was extracted with 100 ml. of ether after the solution had been diluted with 200 ml. of water. Distillation of the wet ether extract left a dark oil which was used, without purification, in the cyclization reaction.

5-Bromo-2-carboxyindole-3-acetic acid

The crude ethyl α-ketoglutaric acid p-bromophenylhydrazone was refluxed for five hours, in a nitrogen atmosphere, with a solution of 20 ml. of concentrated sulfuric acid in 180 ml. of absolute ethanol. The cooled solution was poured into 350 ml. of ice water and the oil which separated was extracted with 150 and 75 ml. portions of ether. The combined ether extracts were washed with half-saturated sodium bicarbonate solution

and distilled. After all the volatile solvents had been removed there remained 12.9 g. of a viscous, red oil. The crude ester was saponified by refluxing 45 minutes with 60 ml. of a 10% ethanolic sodium hydroxide solution. After the solution had cooled, the insoluble disodium salt was filtered off and washed copiously with absolute ethanol to remove a red coloration. The salt was dissolved in 50 ml. of water, boiled with 0.3 g. of Norit A and filtered. Acidification of the filtrate with 10% hydrochloric acid gave 5.6 g. of yellow crystals melting 157-165° (dec.). By concentration and acidification of the mother liquor from which the disodium salt was filtered, 1.3 g. of crystals melting 211-216° (dec.) were obtained. A mixed melting point showed that these two crops of crystals were the same compound. After an unsuccessful attempt to recrystallize the crude product from an ethanol-water mixture, the acid was precipitated as the disodium salt by dissolving in 40% sodium hydroxide and diluting with absolute ethanol. The free acid was recovered from the salt as before. The acid was freed from a tarry contaminant by extracting the acid in a (Sohxlet) extractor overnight with water. The compound separated from the water solution in beautiful needles when seeded. The crystals were filtered off, dried and washed with chloroform to remove a red coloration. This

gave 1.7 g. (0.0057 mole), 13%, of 5-bromo-2-carboxy-indole-3-acetic acid melting 247-248° (dec.). A recrystallization from a dioxane-water mixture did not raise the melting point.

Anal. Calcd. for $C_{11}H_8O_4NBr$: Neut. equiv., 149; N, 4.69

Found: Neut. equiv., 150, 152 (potentiometric); N, 4.48, 4.48

Preparation of Indole-3-acetic Acid from Ethyl γ,γ-Dimethyoxybutyrate (42)

A solution of 20 g. (0.135 mole) of purified ethyl γ,γ-dimethoxybutyrate (b.p. 102-103° at 20 mm.), 16.6 g. (0.135 mole) of phenylhydrazine hydrochloride, 360 ml. of absolute ethanol, and 40 ml. of concentrated sulfuric acid were refluxed in a nitrogen atmosphere for eight hours. The cooled reaction mixture was poured into 1 liter of ice water and the oil extracted with 350 and 250 ml. portions of ether. The combined ether extracts were washed with half-saturated sodium bicarbonate solution and dried over sodium sulfate. Distillation of the ether solution left 21 g. of a yellow oil. This crude ester was purified by vacuum distillation through a short vacuum-jacketed Vigreux column. The fraction distilling 150-155° at 0.1-0.25 mm. and weighing 6.0 g.

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(42) Obtained gratis from the Rohm and Haas Company, Philadelphia, Pa.

was collected as product. The ester was saponified by refluxing twenty minutes with 30 ml. of 10% methanolic potassium hydroxide. Soon after the refluxing began a crystalline (plates) precipitate began forming. These crystals were filtered off and washed with methanol in which they were quite soluble. The salt was dissolved in 20 ml. of water. Acidification of the aqueous solution with 10% hydrochloric acid gave 3.0 g. of indole-3-acetic acid, m.p. 166-167° (dec.). The mother liquor from which the salt was filtered was diluted with 30 ml. of water and distilled until the temperature of the vapor reached 90°. The cooled solution was extracted with 30 ml. of ether, the distillation of which left a trace of crystalline product, having the odor of skatole. Acidification of the aqueous solution with 10% hydrochloric acid gave a white crystalline product, which was filtered off and washed with water. This sample of acid melted 166° (dec.). The total combined yield of the two crops was 4.9 g. (0.028 mole), or 21%. A recrystallization from water (Norit A) gave pure indole-3-acetic acid, m.p. 166-168° (dec.). A mixed melting point with an authentic sample of indole-3-acetic acid was not depressed.

Preparation of 5-Fluoroindole-3-acetic Acid

p-Fluoroaniline

This compound was prepared by the procedure of Schiemann and Pillarsky (43). A mixture of 100 g. (0.71 mole) of p-fluoronitrobenzene and 180 g. (1.52 moles) of 30 mesh granulated tin were placed in a two liter flask, which was equipped with a condenser. The flask was shaken vigorously while 400 ml. of concentrated hydrochloric acid was added in small portions through the condenser. The flask was warmed on the steam bath for one hour. At this time the odor of the starting material could not be detected, and a portion of the reaction mixture did not separate in two phases when diluted with water. The reaction mixture was cooled in an ice bath and made strongly alkaline by the slow addition of a solution of 300 g. of sodium hydroxide in 500 ml. of water. The amine was recovered by exhaustive extraction of the basic solution with ether. An unidentified solid, insoluble in either the water or ether layer, had to be filtered out before the extraction could be carried out efficiently. The combined ether extracts, 1 liter total volume, were dried over sodium sulfate and distilled.

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(43) Schiemann and Pillarsky, Ber., 62, 3041 (1929).

The dark oily residue was purified by vacuum fractionation through a 13 plate column. The fractions were collected as follows:

No.	B.P.°	Press.	Wt., g.
I	77-83	18 mm.	3.5
II	83-84	18 mm.	52.5
III	84-	18-4 mm.	2.0

The yield of pure p-fluoroaniline, fraction II, was 52.5 g. (0.473 mole), or 67%.

p-Fluorophenylhydrazine hydrochloride

This preparation was patterned after the procedure described by Schiemann and Winkelmuller (44) for the preparation of the free base. A solution of p-fluorophenyldiazonium chloride, prepared by the diazotation, at 0°, of 40 g. (0.36 mole) of p-fluoroaniline in 145 ml. of 6 M hydrochloric acid with a solution of 27.2 g. (0.394 mole) of sodium nitrite in 73 ml. of water, was poured into an ice cold, saturated solution of 240 g. (0.95 mole) of sodium sulfite heptahydrate. The resulting orange solution was shaken and diluted slowly with 110 ml. of concentrated hydrochloric acid. The

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(44) Shiemann and Winkelmuller, Ber., 66, 729 (1933).

now yellow solution was heated on the steam bath for two hours. The solution became red soon after the heating was commenced and faded to a pale orange as the heating continued. Fifty ml. of acetic acid was added to the hot solution followed by approximately 5 g. of powdered zinc, added in small quantities, in an unsuccessful attempt to completely decolorize the solution. An insoluble red solid was filtered off and the filtrate made strongly acid by the addition of 220 ml. of concentrated hydrochloric acid. The hydrochloride began separating immediately. After the solution had cooled overnight in the ice box the well-formed, pink-tinted crystals were filtered off and washed with 160 ml. of 10% hydrochloric acid and with 300 ml. of anhydrous ether. The yield of the p-fluorophenylhydrazine hydrochloride was 17.6 g. (0.108 mole), or 30%. The hydrochloride had no definite melting point.

An attempt to recover more product by alkalization of the mother liquor with subsequent extraction of the free base with ether gave only 5 g. of crude p-fluoroaniline. The red solid which was filtered from the acid sulfite solution weighed 9.7 g. After two recrystallizations from benzene the product melted 150° (dec.). A qualitative analysis showed that the compound contained sulfur. This suggests that the compound is p-fluorophenyldiazosulfonic acid. The presence of this latter

product indicates that sufficient sodium sulfite to reduce the diazonium salt was not present.

5-Fluoroindole-3-acetic acid

A solution of 20 g. (0.135 mole) of ethyl γ, γ-dimethoxybutyrate, 17.6 g. (0.108 mole) of p-fluorophenylhydrazine hydrochloride, 360 ml. of absolute ethanol and 40 ml. of concentrated sulfuric acid were refluxed in a nitrogen atmosphere for eight hours. The cooled solution was poured into 1 liter of ice water and the oil was extracted with 350, 250, and 150 ml. portions of ether. The combined ether extracts were washed with half-saturated sodium bicarbonate solution, dried over sodium sulfate and distilled. The oily residue was transferred to a 50 ml. flask and vacuum distilled through a short vacuum-jacketed Claisen still head. The fractions were collected as follows:

No.	B.P.°	Press.	Wt., g.
I	62-110	0.5-0.6 mm.	0.5
II	150-175	0.4 mm.	14.2
Distilling residue			6.0 (estimated)

The crude ester, fraction II, was saponified by refluxing twenty minutes with 60 ml. of 10% methanolic potassium hydroxide. The alkaline solution was diluted

with 50 ml. of water and distilled until the temperature of the vapor was 96°. The cooled solution was extracted with 30 ml. of ether, the distillation of which left no residue. The aqueous solution was heated to boiling with 0.5 g. of Norit A and filtered. Acidification of the still dark filtrate with 10% hydrochloric acid gave 11.0 g. (0.57 mole) of tan crystals melting 138-139°. The yield, based on the p-fluorophenylhydrazine hydrochloride, was 53%. A recrystallization of the acid from water (Norit A) raised the melting point to 138-140°.

Anal. Calcd. for $C_{10}H_8O_2NF$: Neut. equiv., 193.18; N, 7.24

Found: Neut. equiv., 192 (potentiometric); N, 7.01, 7.12

Determination of the Plant Growth Activity of Substituted Indole-3-acetic Acids

Method

The Pea Test of Went (45, 46) was employed to determine the hormonal activity of the substituted indole-

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(45) van Oberbeek and Went, Botan. Gaz., 99, 22 (1937). Original not seen. Cited by Findlay and Dougherty, J. Biol. Chem., 183, 361 (1950).

(46) Went and Thimann, "Phytohormones", MacMillan Company, New York, N.Y., 1937.

3-acetic acids.

Alaska peas (obtained from the Farmer Seed and Nursery Co.) were sterilized by washing with 95% ethanol. The peas were then washed well with distilled water. After soaking in water for six hours they were planted in moist sand and grown in total darkness at room temperature. At age seven to nine days they had reached a height of 10-12 cm. and had developed two nodes, each bearing a scale and the uppermost bearing a leaf. Those plants in which the internode between this leaf and the terminal bud had reached less than 5 mm. were selected. The top was cut off just below this leaf and the stem split centrally lengthwise for a distance of about 3 cm. with a razor blade. The split section was then cut off a few mm. below the slit and washed for an hour in distilled water. The split sections were placed in petri dishes containing 20 ml. of the test solution. Five or six split sections were placed in each petri dish. The plates were stored in the dark overnight and shadowgraphed on bromide paper. According to Went, the curvatures reach a maximum after six hours and remain unchanged.

If no plant growth regulator is present the two longitudinal sections will curve away from each other, while if an active substance is present in the proper

concentration the free ends will bend inward. The degree of inward bending is proportional to the activity of the compound and to the concentration.

Stock solutions of the compounds to be tested were prepared by weighing out 10 mg. samples of the acids on a semimicrobalance, adding an equivalent amount of sodium hydroxide and diluting to 250 ml. Since Went reported that the presence of some metal ions inhibit the curvatures, glass-distilled water was used in making up solutions and for rinsing the glassware.

Results

The sodium salts of indole-3-acetic, 4-chloroindole-3-acetic, 5-chloroindole-3-acetic, 6-chloroindole-3-acetic, 7-chloroindole-3-acetic, 4- and 6-chloroindole-3-acetic (eutectic mixture), 5,7-dichloroindole-3-acetic, 5-fluoroindole-3-acetic, and 2-carboxyindole-3-acetic acid were tested in serial dilutions in range of 10 to 0.25 mg. of the acid per liter. The results are summarized in Table I.

Table I

Plant Growth Activity of Substituted Indole-3-acetic Acids

Substance[1]	Concentration in mg. of acid per liter						
	5	2	1	0.5	0.25	0.1	0.05
Indole-3-acetic acid	+	+	+	+	+	+	+
4-Chloroindole-3-acetic acid	+	+	+	+	+	+	-
5-Chloroindole-3-acetic acid	+	+	+	+	+	+	-
6-Chloroindole-3-acetic acid	+	+	+	+	+	+	-
7-Chloroindole-3-acetic acid	+	+	+	+	+	?	-
4- and 6-Chloroindole-3-acetic acids (eutectic mixture)	+	+	+	+	+		
5,7-Dichloroindole-3-acetic acid	+	-	-	-	-		
2-Carboxyindole-3-acetic acid	-	-					
5-Fluoroindole-3-acetic acid	+	+	+	+	+	+	-

[1]The sodium salts were used in the tests.

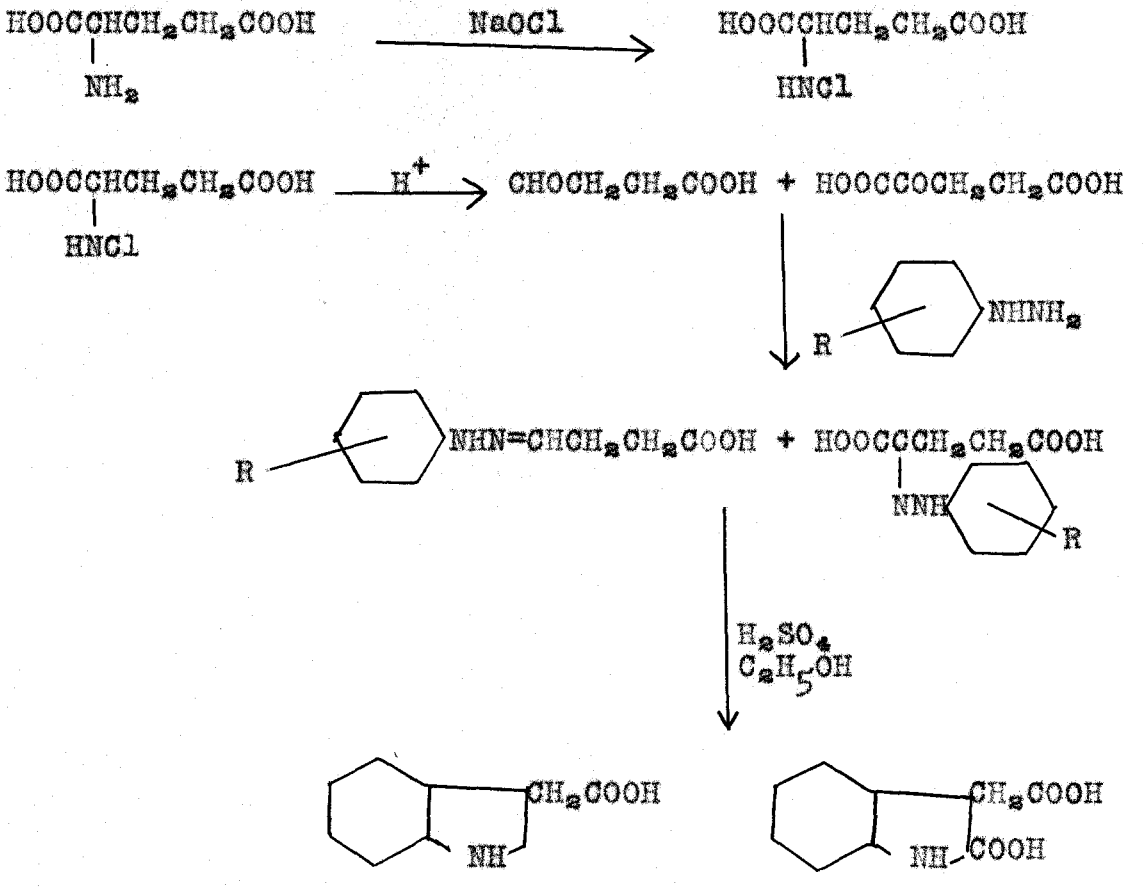
Although the Pea Test for hormone determination is only roughly quantitative the results indicate that substitution of halogens in the benzene ring has little beneficial effect on the activity of the compound in the Went Pea Test. This is in contrast to the 2-methylindole-3-acetic acid series which were studied by Stevens (1) and the phenoxyacetic acid series which were studied by Hitch-

cock (3). It is worthy of note that all the monohalogen compounds tested had very nearly the same activity. It is not unusual that the 2-carboxy derivative has no activity as the 2-methyl derivative has only 20% of the activity of indole-3-acetic acid and the 2-ethyl derivative is almost completely inactive (46).

DISCUSSION AND CONCLUSIONS

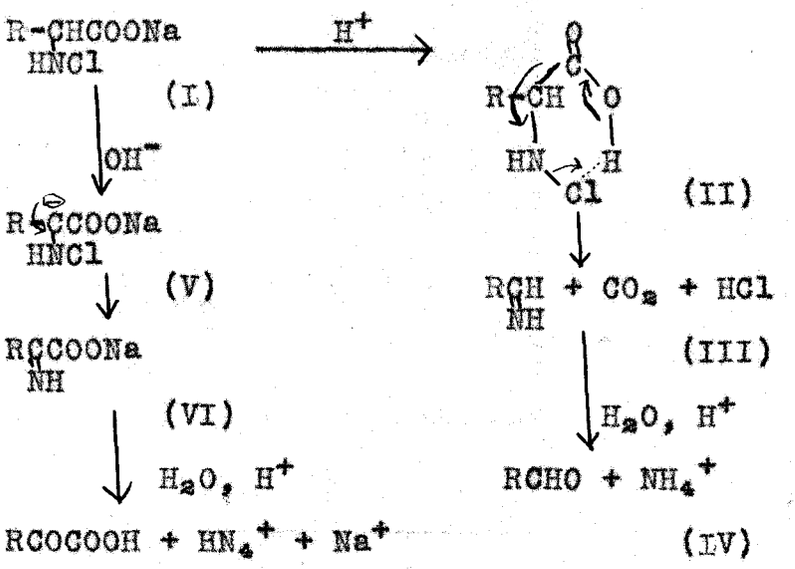
Chemical

An economical conversion of glutamic acid to indole-3-acetic acid and to derivatives of indole-3-acetic acid with substituents in the benzene ring has been brought under control. The chemistry of the process is illustrated in the equations below:



In addition to the succinaldehydic acid formed by the decomposition of the α-N-chloroaminoglutaric acid there was also formed, as a by-product, a small amount of α-ketoglutaric acid. The α-ketoglutaric acid phenylhydrazones were formed along with the principal product. Cyclization of these α-ketoglutaric acid phenylhydrazones gave the 2-carboxyindole-3-acetic acids.

Since α-keto acids are not decarboxylated under the conditions employed in the decomposition of the α-N-chloroaminoglutaric acid, the α-keto acid can not be an intermediate in the formation of the aldehyde. In order to account for the products formed in the reaction mixture the following mechanism for the decomposition of the α-N-chloroamino acid is proposed: (47)



(47) Acknowledgement is given to Dr. G. S. Hammond, Mr W. T. Meikle, and Mr. T. C. Myers for assistance in formulating the mechanism.

Acidification of the sodium salt of the α-N-chloro-amino acid (I) gives the free acid which immediately forms the six membered chelated ring (II). If the electron shifts indicated by the curved arrows occur, then the molecule can decompose to form three smaller stable molecules. The imino compound (III) then hydrolyzes to form the aldehyde (IV). The yield of the α-keto acid appears to be proportional to the time elapsed between the formation of and acidification of the α-N-chloro-amino acid. This suggests that the formation of the α-keto acid occurs through the loss of a proton from (I) to form the carbanion (V), which immediately or simultaneously stabilizes itself by the loss of a chloride ion to form the α-imino acid salt (VI). This imino group then hydrolyzes to the carbonyl to form the α-keto acid (VII).

By using the method described above for the conversion of glutamic acid to indole-3-acetic acids, the 5- and 7-chloro, 5,7-dichloro, and 5-methylindole-3-acetic acids have been prepared. Cyclization of the succinaldehydic acid m-chlorophenylhydrazone was expected to yield a mixture of 4-chloroindole-3-acetic acid and 6-chloroindole-3-acetic acid. Two products were obtained as expected. The principal product was an eutectic mixture of the 4- and 6-chloroindole-3-acetic acids while

the other product was a small amount of 6-chloroindole-3-acetic acid.

To solve the problem of identifying the isomers obtained in the cyclization of the meta substituted phenylhydrazone, both the 4- and 6-chloroindole-3-acetic acids were synthesized by unambiguous methods. The corresponding chloroindoles were prepared by condensing the properly substituted 2-nitrotoluenes with ethyl oxalate to yield the 2-nitrophenylpyruvic acid derivatives, from which the chloroindole-2-carboxylic acids were formed by a reductive cyclization with ferrous hydroxide. Decarboxylation of these acids gave the chloro-substituted indoles. After an unsuccessful attempt at the synthesis of the 4-chloro acid through the reaction of 4-chlorogramine with sodium cyanide, both the 4-chloroindole-3-acetic acid, and the 6-chloroindole-3-acetic acids were successfully prepared by condensing the corresponding indolylmagnesium iodide complexes with chloroacetonitrile with subsequent hydrolysis of the resulting nitriles to the acids.

Several of the 2-carboxyindole-3-acetic acids were obtained either by cyclization of the α-ketoglutaric acid phenylhydrazines, which in turn resulted as by-products in the preparation of the succinaldehydic acid phenylhydrazones. They were in some cases obtained as diethyl

esters, by the Japp-Klingemann reaction between ethyl α-acetoglutarate and a phenyldiazonium salt.

The 2-carboxyindole-3-acetic acids prepared by these methods included the 7-chloro, 5- and 7-methyl, and 5-bromo derivatives as well as the unsubstituted parent compounds. No attempt was made to cyclize the α-ketoglutaric acid m-chlorophenylhydrazone; attempts to cyclize the α-ketoglutaric acid 2,4-dichlorophenylhydrazone failed.

An alternate synthesis of indole-3-acetic acid and some of its derivatives, employing ethyl γ, γ-dimethoxybutyrate in lieu of glutamic acid, has been worked out. The ethyl succinaldehydate phenylhydrazone was prepared from the acetal and the phenylhydrazine and cyclized to the ethyl indole-3-acetate in one operation. Saponification of the ester then yielded the free acid. Indole-3-acetic acid and 5-fluoroindole-3-acetic acid have been prepared in this way and the method appears to be applicable to very general use.

Physiological

The substituted indole-3-acetic acids prepared were tested for hormone activity by the Went Pea Test (45, 46). The results of these tests showed, in contrast to the 2-methylindole-3-acetic acid (1) and phenoxyacetic acid (3)

series, that substitution of one halogen into the benzene ring had little effect on the activity of the compound. The 5,7-dichloroindole-3-acetic acid was considerably less active than any of the monohalogen derivatives or the parent compound, indole-3-acetic acid. Since the 2-carboxyindole-3-acetic acid showed no activity, the substituted 2-carboxyindole-3-acetic acids were not tested.

The low order of activity of the 2-substituted indole-3-acetic acids can probably be attributed to steric hindrance as the activity of the compound appears to be inversely proportional to the size of the group. The order of decreasing activity has been observed to be 2-methylindole-3-acetic acid, 2-ethylindole-3-acetic acid (46), and 2-carboxyindole-3-acetic acid. Since the 1-methylindole-3-acetic acid (46) is also less active than indole-3-acetic acid it would appear that any substituent in the pyrrole ring will decrease the activity.

On the other hand, it would appear that the activity is not greatly influenced by the presence of a single atom or group on the benzene ring. This is supported by evidence, presented in this thesis, that the activity of all the compounds with a single halogen substituted in the benzene ring have approximately the same activity as the parent indole-3-acetic acid in the Went Pea Test. This hypothesis is also born out by the fact that the

5-methylindole-3-acetic acid (46), and the 5- and 6-methoxyindole-3-acetic acids (9) have about the same activity as the unsubstituted acid. The decreased activity of the 7-methoxyindole-3-acetic acid (9) and of the 5,7-dichloroindole-3-acetic acid do not conform to this generality.

SUMMARY

1. A practical method for the preparation of indole-3-acetic acid from glutamic acid has been developed.

2. This method has been found to be especially applicable to convenient synthesis of indole-3-acetic acids substituted in the benzene ring.

3. α-Ketoglutaric acid has been isolated, in the form of substituted phenylhydrazones, from the decomposition of α-N-chloroaminoglutaric acid.

4. A mechanism embracing the α-ketoglutaric acid by-product has been proposed for the conversion of the glutamic acid to the intermediate succinaldehydic acid.

5. All the derivatives of indole-3-acetic acid containing a single chlorine atom in the benzene ring have been described. The 5,7-dichloro, 5-fluoro, and 5-methyl derivatives have also been prepared.

6. Preliminary Pea Tests have shown that all the monohalogen derivatives tested have approximately the same activity as the unsubstituted indole-3-acetic acid, while the dichloro compound is less active.

7. Several of the derivatives of 2-carboxyindole-3-acetic acid have been described. These include the 5- and 7-chloro and the 5-bromo derivatives as well as

the parent 2-carboxyindole-3-acetic acid.

8. Pea Tests were carried out for the unsubstituted 2-carboxyindole-3-acetic acid, which exhibited no activity.

9. An alternate synthesis for the preparation of indole-3-acetic acid itself and for derivatives substituted in the benzene ring, employing ethyl γ, γ-dimethoxybutyrate in lieu of glutamic acid, has been described.

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