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Some substituted 3-indoleacetic acids

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Iowa State College

NOTE TO USERS

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SOME SUBSTITUTED 3-INDOLEACETIC ACIDS

by

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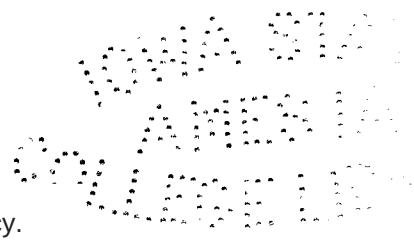

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INTRODUCTION

The present interest in substituted derivatives of 3-indoleacetic acid, a naturally occurring plant growth hormone of high activity, is in part due to a significant discovery of Hitchcock (124). He found that the plant growth activity of phenoxyacetic acid was greatly enhanced by the substitution of chlorine atoms into the benzene nucleus. o-Chlorophenoxyacetic acid was twenty, p-chlorophenoxyacetic acid was eighty, and 2,4-dichlorophenoxyacetic acid was six hundred and fifty times as effective as the parent compound. He also found that derivatives of benzoic acid were active after the proper substitution of chloro, bromo and nitro groups. Benzoic acid itself possesses no activity.

A survey of the literature showed that no 3-indoleacetic acids with nitro or chloro groups substituted into the aromatic ring had been prepared. One of the objectives of the present work was to prepare and test the plant growth activity of some indoleacetic acids with groups that had been shown to be active in other series substituted in the benzene part of the ring. Since the 2,4-dichloro- derivative of phenoxyacetic acid was so effective, the preparation of a 3-indoleacetic acid compound with two chlorine atoms meta to each other was attempted.

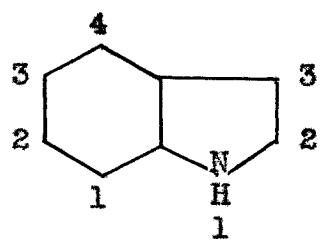
3-(2-Methylindole)-acetic acid also shows some plant growth activity and some of its substituted derivatives were also prepared.

One of the simplest methods for the preparation of indole derivatives is the Fischer Synthesis (26) in which a properly substituted phenylhydrazone is cyclized. For the preparation of 3-indoleacetic acid, the phenylhydrazone of β-formylpropionic acid would be used.

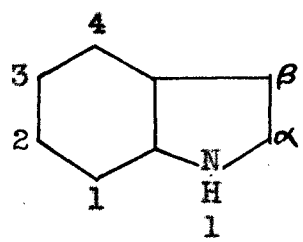
Langheld (78) prepared β-formylpropionic acid in small quantities from glutamic acid. This reaction is of special interest because glutamic acid is readily available at reasonable cost as a by-product of the corn refining industry. The reaction was studied further to determine the feasibility of converting glutamic acid to 3-indoleacetic acid.

NOMENCLATURE

The nomenclature of indole derivatives in the literature is varied and confusing since several different systems are used in designating substitutions. Indole has the formula C_8H_7N and its structure as usually written with the three common methods of numbering is shown by formulas I, II and III.



I



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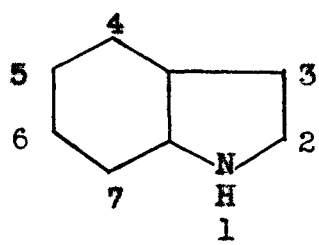


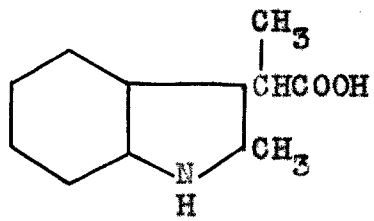
III

In the system using formula I, substitution in the benzene ring is shown by the symbol Bz-1, Bz-2, etc., and in the pyrrole ring by Pr-1, etc.

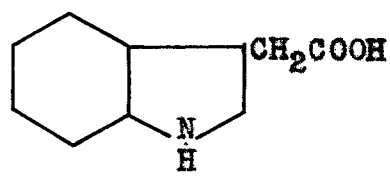
The second system which uses α and β for the carbons in the pyrrole ring designates substitution on the nitrogen by (N-). This system is particularly confusing in naming compounds like tryptophan and indole acids in which the Greek letters are also used to show substitution in the chain.

This paper will use the third method in which substitution in the indole group will be indicated by numbers and

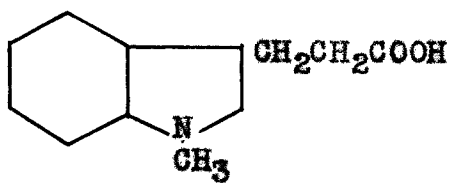
Greek letters to show the position of the indole group in the acid chain, as for example:



α-3-(2-methylindole)-propionic acid



3-indoleacetic acid



β-3-(1-methylindole)-propionic acid

HISTORICAL

Plant Growth Hormones

There are a number of excellent reviews on plant hormones available that cover both the chemical and botanical aspects of the subject. In this survey the work on 3-indoleacetic acid and other indole acid derivatives will be stressed. For a more comprehensive discussion, the reviews by du Buy and Nuernbergk (12), Erxleben (21), von Guttenburg (36, 37, 38, 39, 40), Heyn (47), Jost (57), Kögl (66, 67, 68), Haagen-Smit (41), Stiles (98), Thimann (103, 106), Went, F. W. (114, 115, 116), Went, F. A. F. C. (111), Boysen Jensen, translated and extended by Avery and Burkholder (11), and Van Overbeek (110) should be consulted.

The history of plant growth hormones began in 1913 when Boysen Jensen (9, 10) showed that the tip of the oat (Avena sativa) primary leaf sheath or coleoptile contains a substance that migrates toward the base of the coleoptile and controls its growth. Coleoptile tips of Avena seedlings which had been grown in the dark were cut off and then replaced on the stumps over a layer of gelatin. Unilateral illumination of the tips alone resulted in the curvature of the entire coleoptile toward the light. This showed that light in some way affected the active material on the

illuminated side while that on the dark side diffused through the gelatin. This work was confirmed by Paál (87), who also placed the coleoptile tip on one side of the coleoptile stump and obtained a curvature away from the side the tip was applied to. The entire experiment was conducted in the dark.

Final proof for the existence of such a substance was obtained by Went (112) in 1928. He placed Avena coleoptile tips on agar, allowing the active substance to diffuse into it, and than applied small blocks of this agar on one side of decapitated Avena coleoptiles. He noted that these blocks were active in promoting curvatures away from the side to which they were applied. This technique became the basis for the Avena test for plant hormones which Went developed. At first it was thought that the degree of curvature was proportional to the amount of growth substance in the agar block, but after further study the curvature was found to be proportional to the concentration (117).

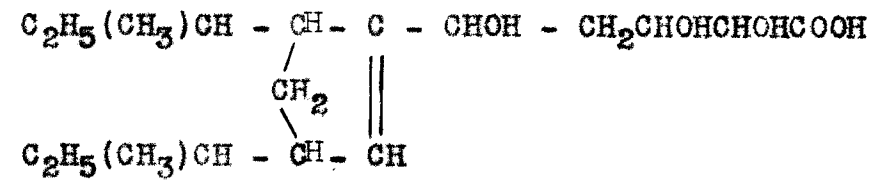
The first chemical study of the active substance was made by Nielson (84) in 1930 who found an active substance, extracted from the medium upon which Rhizopus suinus had been grown, to be ether-soluble. Dolk and Thimann (18) observed that the substance was ether-soluble only in acidified solutions. They determined that the substance was an acid with an ionization constant of 1.8×10^{-5}, easily

destroyed by peroxides and therefore unsaturated.

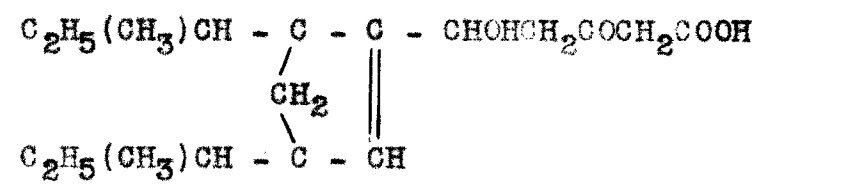
Kögl, Haagen-Smit and Erxleben (74, 75) discovered that the substance was present in large amounts in human urine. From the sodium bicarbonate fraction of the ether extract of urine concentrate, they isolated an acid, $C_{18}H_{32}O_5$, and its lactone. Both forms were active in the Avena test. A 10° curvature was obtained using 3 x 10^{-8} mg. which was defined as one Avena-Einheit, AE. The value for this unit was later changed to 2 x 10^{-8} mg. (65). The acid was called auxin a. Later another acid was isolated from malt and corn germ oil which was isomeric with the lactone of auxin a (73). This new substance was called auxin b.

The structure of the auxins was elucidated by Kögl and co-workers at Utrecht, Holland (70, 71, 72, 75). The formulas as established by degradation and synthesis were:

auxin a — auxentriolic acid



auxin b — auxenolonic acid



In an attempt to work up larger volumes of urine using a charcoal absorption method they isolated another very active substance which they called heteroauxin. This was found to be 3-indoleacetic acid (76). However β-3-indole-

propionic acid, 2-indolecarboxylic acid, and 3-indolecarboxylic acid were found to be inactive using the Avena test.

The active substance in Avena coleoptiles was shown to be auxin a by the molecular weight determinations of Went (112), and by the stability of the substance to acid and its destruction by alkali as found by Kögl and co-workers (76). On the other hand, the substance from molds was proven to be 3-indoleacetic acid by Thimann (104).

In addition to their growth promoting function which is caused by cell elongation, the three naturally occurring auxins were shown to exert other influences upon plants. Inhibition of root growth, the formation of roots upon cuttings, bud inhibition, callus development and stem swelling, cambial growth and cell division were attributed to these substances (117). The setting of fruit without pollination or parthenocarpy, modification of organs (100), and herbicidal action (85) are recently discovered responses caused by plant growth substances.

In 1935 Hitchcock (48) caused epinasty of leaves (downward bending), and bending and swelling of intact tomato, tobacco, and African marigold plants by the local application of 3-indoleacetic and β-3-indolepropionic acids. This caused him to make the statement, "The fact that one homologue of heteroauxin was found to be active in causing certain formative responses indicates that possibly other indole derivatives and perhaps other unrelated chemicals might in-

duce one or more of these same responses."

Zimmerman and Wilcoxon (128) tested twelve indole derivatives and some related compounds and found that only 3-indoleacetic and γ-3-indolebutyric acids were active, while β-3-(2-carboxyindole)-ethyl phenyl ether, β-3-(2-carboxyindole)-propionic, β-3-(2-carboxy-5-methoxyindole)-propionic, β-3-(2-carboxy-6-methoxyindole)-propionic, β-3-(2-carboxy-7-methoxyindole)-propionic, β-3-(5-methoxyindole)-propionic, β-3-(6-methoxyindole)-propionic, β-3-(7-methoxyindole)-propionic, α-3-indolebutyric, 3-indolesuccinic, and α-3-(2-carboxyindole)-butyric acids were not active.

Another homologue of heteroauxin was added to the list of active compounds when Zimmerman and Hitchcock (121) noted that Δ-3-indolevaleric was active.

Thirty-four compounds, mostly derivatives of the auxins, were studied by Kögl (66, 67) and Kögl and Kostermans (77). The activities for the indole compounds are given in Table 1.

Bauguess (5) found that β-3-indolepyruvic, β-3-indole-α-oximinopropionic, and β-3-indoleacrylic acids were active in root initiation, stem bending and bud inhibition on tomatoes, marigolds and stocks. The activity of these acids was about the same as that of β-3-indolepropionic acid, but less than that of 3-indoleacetic acid. Indolelactic acid was found to be inactive.

Table 1

Activities of Indole Derivatives[1]

Substance	Activity in AE/gram
3-indoleacetic acid	25,000,000,000
methyl 3-indoleacetate	10,000,000,000
ethyl 3-indoleacetate	3,000,000,000
n-propyl 3-indoleacetate	1,000,000,000
isopropyl 3-indoleacetate	100,000,000
3-(2,3-dihydroindole)-acetic acid	inactive
methyl 3-(2,3-dihydroindole)-acetate	inactive
3-(1-methylindole)-acetic acid	30,000,000
ethyl 3-(1-methylindole)-acetate	inactive
3-(2-methylindole)-acetic acid	125,000,000
methyl 3-(2-methylindole)-acetate	inactive
3-(2-ethylindole)-acetic acid	inactive
3-(5-methylindole)-acetic acid	1,500,000,000
methyl 3-(5-methylindole)-acetate	1,200,000,000
3-(2,5-dimethylindole)-acetic acid	inactive
β-3-indolepropionic acid	inactive
3-indolecarboxylic acid	inactive
2-indolecarboxylic acid	inactive
α-3-indolepropionic acid	5,000,000,000
β-3-indolelactic acid	inactive
3-indolepyruvic acid	200,000,000
β-3-indole-α-aminopropionic acid	inactive

[1]Reported by Kögl (66, 67) and Kögl and Kostermans (77).

Haagen-Smit and Went (42) examined a number of compounds by the Avena test and reported that 3-indolepyruvic acid had 20 per cent the activity of 3-indoleacetic acid, while methyl 3-(2-methylindole)-acetate was inactive. A pea test based upon the curvature produced upon pieces of etiolated pea seedlings that have been slit longitudinally was developed by Went (113). Approximately the same results were obtained by this test for the compounds mentioned above, and in addition 3-(2-ethylindole)-acetic acid and indolecarbonic acid were found to be inactive.

The activity of 3-indoleacetic acid and two analogues, 3-indeneacetic acid and l-coumarylacetic acid, were compared by Thimann (105). The analogues produced cell elongation in etiolated Avena sections, produced roots, inhibited root elongation, and were therefore true growth promoting substances, but l-coumarylacetic acid was not active in the Avena curvature test. The activity of 3-indeneacetic acid is about the same as 3-indoleacetic acid, but it is transported more slowly in the plant. Thimann pointed out that the transport ability and the growth promoting ability are separate properties and that the failure of l-coumarylacetic acid to produce curvature in the Avena test may be explained because it is transported in all directions in the plant as opposed to the polar transport of 3-indoleacetic acid.

Glover (30) reported that skatole, 3-methylindole, had slight activity.

The methyl esters of 3-indoleacetic, β-3-indolepropionic and γ-3-indolebutyric acids were found by Zimmerman, Hitchcock and Wilcoxon (126) to be more effective than the corresponding acids in causing bending of tomato leaves and stems.

Zimmerman and Hitchcock (122) found that the salts potassium, sodium, ammonium, trimethylammonium and tetraammonium 3-indoleacetate, potassium and sodium β-3-indolepropionate, sodium, potassium, trimethylammonium and tetramethylammonium γ-3-indolebutyrate were as effective as their corresponding acids in accelerating growth locally (bending) and inducing root formation.

Manske and Leitch (81) prepared a number of compounds which were tested for activity by Zimmerman of the Boyce Thompson Institute. γ-3-Indolebutyric, Δ-3-indolevaleric, β-3-(5-methylindole)-propionic, and 1,3-indylenediacetic acids were found to be effective.

A modification of the Avena test which proved to be about ten times as sensitive was developed by Skoog (96) who found that tryptophan and indoleethylamine became activated about two hours after application. He suggested that these compounds, together with indolepyruvic acid, are precursors of 3-indoleacetic acid.

The Avena test was employed by Davies, Atkins and Hudson (17) to test several indole derivatives that they prepared. ω-Skatolemalonic acid, 3-ethylindole, 3-indolecarboxylic acid and β-3-indolepropionic acid were all found to be inactive.

Kögl (69) resolved α-3-indolepropionic acid and discovered that the activity of the (+) antipode was 48 x 10^9, the (-) antipode 1.6 x 10^9, and the racemic mixture 23 x 10^9 A.V. per gram.

In an attempt to correlate growth activity with structure Koepfli, Thimann and Went (64) studied a large number of compounds including a number of indole derivatives. The pea test was used for this study because the transportability of the substance is not as important as it is in the Avena test. The active indole compounds were: 3-indoleacetic acid, 100%; β-3-indolepropionic acid, 100%; α-3-indolepropionic acid, 100%; γ-3-indolebutyric acid, 100%; and Δ-3-indolevaleric acid, 50%.

Inactive indole derivatives included: skatole, indoxyl, 3-indolecarboxylic acid, 3-indolesuccinic acid, 2-indolecarboxylic acid, β-3-(2-carboxyindole)-propionic acid, β-3-(2-carboxyindole)-butyric acid, 2-(3-hydroxyindole)-carboxlic acid, β-3-(5-methoxyindole)-propionic acid, β-3-(6-methoxyindole)-propionic acid, β-3-(7-methoxyindole)-propionic acid, 1-acetylindoxyl, and isatin.

As a result of their study they listed five minimum structural requirements for an active growth substance:

1. A ring system

2. Unsaturation in the ring

3. A side chain

4. A carboxyl at least one carbon atom removed from the ring

5. A very definite space relationship between the ring and the carboxyl

All the known active compounds had a homocyclic or heterocyclic five or six membered ring, and no aliphatic compounds were known that had activity. In addition, when auxin a. auxin b, or 3-indoleacetic acid were hydrogenated all activity disappeared. If the ring were substituted sometimes an active product was obtained, while in some cases activity was destroyed. Haagen-Smit and Went (42) found that substituting a methyl group in the 1, 2, or 5 position of indoleacetic acid did not make the compounds inactive, but an ethyl group in the 2 position did. A methoxy group in the 5, 6, or 7 position of β-3-indolepropionic acid made the substance inactive (64).

The known compounds all have a carboxyl in the side chain or could be hydrolyzed to carboxylic acids. The activity reported by Glover (30) for skatole was shown to have been due to an impurity, and all other indoles lacking a carboxyl were inactive. There seemed to be no limitation on

the length of the side chain carrying the carboxyl group except that it had to be at least one carbon removed from the ring. More than one carboxyl seemed to destroy activity, however Manske and Leitch (81) found 1,3-indylenediacetic acid was active.

The space configuration seems to be very important. Cis-isomers of several substituted cinnamic acids were active while the trans- modifications were not. The inactivity of several dicarboxylic acids and the difference in activity between 3-(2-methylindole)- and 3-(2-ethylindole)-acetic acids seemed to substantiate this. Additional proof was the difference in activity of the enantiomorphs of α-3-indolepropionic acid (69).

Zimmerman, Hitchcock and Wilcoxon (127) applied vapors of various substances to plants and obtained responses similar to those obtained with solutions. Methyl 3-indole-acetate, 3-indoleacetic acid, ethyl 3-indoleacetate, 3-(2-methylindole)-acetic acid, β-3-indolepropionic acid, methyl β-3-indolepropionate, γ-3-indolebutyric acid, methyl γ-3-indolebutyrate, and ethyl γ-3-indolebutyrate were all active.

Another indole derivative was added to the list of active vapors by Zimmerman and Hitchcock (123) who found methyl α-trimethylamino-β-(3-indole)-propionate iodide to be active.

Gustafson (34) observed that potassium 3-indoleacetate

was more active than the acid itself. The entire indole ring does not seem to be necessary since he found several pyrrole acids to be active.

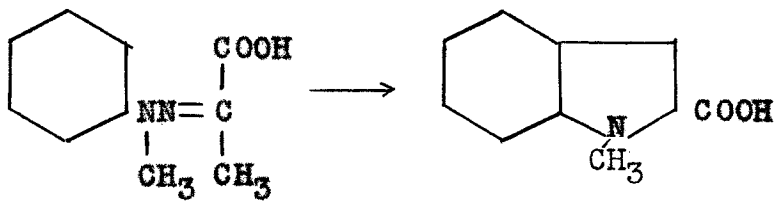
In 1942 Avery, Berger and Shalucka (2), using the deseeded Avena test, found the following order of activity for several indole derivatives: 3-indoleacetic acid, 100; potassium 3-indoleacetate, 94; methyl 3-indoleacetate, 62; ethyl 3-indoleacetate, 29; 3-indoleacetamide, 0-.4; γ-3-indolebutyric acid, 4-5; potassium γ-3-indolebutyrate, 4-5; methyl γ-3-indolebutyrate, 4-5; and ethyl γ-3-indolebutyrate, 4-5.

It is evident from the preceding paragraphs that there seems to be no good correlation between workers using different test methods. Several investigators, Zimmerman and Hitchcock (122), Thimann and Schneider (107), and Avery, Berger and Shalucka (2) have recognized and discussed this disagreement in results.

Gustafson (35), in an attempt to interrelate some of the results found in the literature, used seven different methods and found that none of the compounds was equally effective in all the tests. He concluded that the different tests are each suited for the measurement of specific activities and therefore care should be used in choosing a test method.

Preparation of Indole Acids

The majority of the indole derivatives have been prepared by the interesting synthesis discovered by Fischer (26,27). In the course of an investigation on the phenylmethylhydrazone of pyruvic acid, the material was boiled with 10% hydrochloric acid. A substance was isolated in about 5% yield which proved to be 2-(1-methylindole)-carboxylic acid. The reaction therefore involved the elimination of a molecule of ammonia.



Further investigation by Fischer (25) showed that the reaction was a general one and that zinc chloride was a better catalyst. Among the indole compounds which he prepared were 2-indolecarboxylic acid, 3-(2-methylindole)-acetic acid, 3-(1,2-dimethylindole)-acetic acid, 3-(1,2-dimethylindole)-carboxylic acid, and 2-(1-ethylindole)-carboxylic acid.

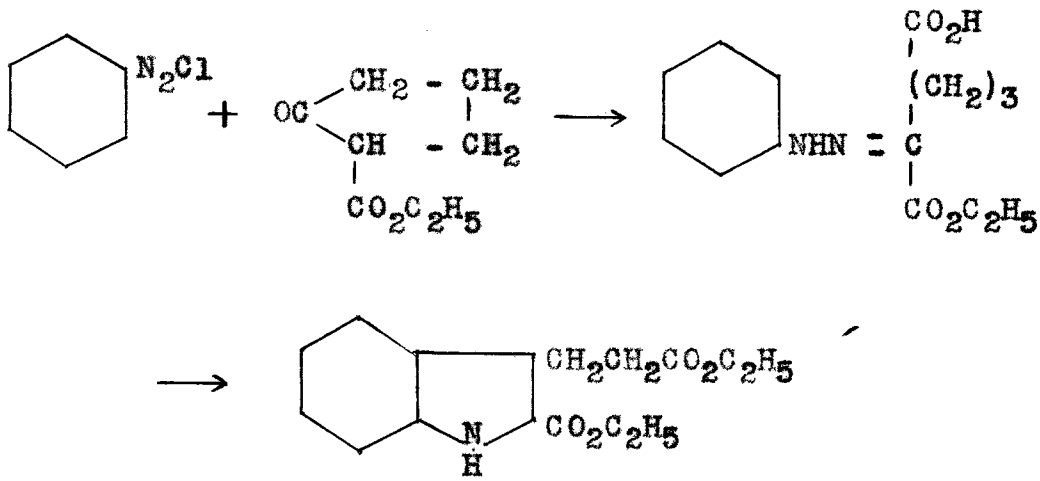
The Fischer synthesis has undergone many modifications particularly in the nature of the acidic reagent used. The

early modifications are discussed by Van Order and Lindvall (109) in their review on indoles. The most widely used methods are those of Fischer (25), using zinc chloride, and Wislicenus and Arnold (119), using alcoholic sulfuric acid. Other reagents that have been used are stannous chloride, alcoholic hydrogen chloride, concentrated sulfuric acid, nickel chloride, copper chloride (109), glacial acetic acid, concentrated hydrochloric acid, hydrogen bromide in glacial acetic acid (51), and boron trifluoride (97).

Hartley and Dobbie (45) prepared 2-(1-methylindole)-carboxylic acid from the methylphenylhydrazone of pyruvic acid by Fischer's original procedure.

3-Indoleacetic acid was synthesized by Ellinger (19) in 1904 from the phenylhydrazone of β-formylpropionic acid. Alcoholic sulfuric acid was used as the condensing agent. The same procedure was used to prepare α-3-indolepropionic acid from β-formylisobutyric acid, and β-3-indolepropionic acid from γ-formylbutyric acid (20).

Kalb, Schweizer and Schimpf (58) introduced the Japp-Klingemann (55) reaction for the preparation of the required phenylhydrazones, which made the preparation of the phenylhydrazine unnecessary. Ring closure was effected with alcoholic sulfuric acid.



The dicarboxylic acid, β-3-(2-carboxyindole)-propionic acid, was obtained from the ester and could be decarboxylated easily to β-3-indolepropionic acid.

Several indolepropionic acids which were substituted in the benzene nucleus were prepared by Kalb, Schweizer, Zellner, and Berthhold (59) from the properly substituted aniline derivatives and the above procedure. The acids prepared were: β-3-(2-carboxy-5-nitroindole)-propionic acid, β-3-(2-carboxy-5-iodoindole)-propionic acid, β-3-(2-carboxy-4,5,6-triiodo-indole)-propionic acid, and β-3-(2-carboxy-5-methoxy-4,6-diiodoindole)-propionic acid.

The Japp-Klingemann-Fischer combination was used by a number of investigators to produce indole acids. Keimatsu and Sugasawa (60, 61) obtained 2-(3-methylindole)-carboxylic, 3-(2-carboxyindole)-acetic, β-3-(2-carboxyindole)-propionic, and β-3-indolepropionic acid. Barrett, Perkin and Robinson (4) prepared β-3-(2-carboxy-5-methoxyindole)-propionic,

β-3-(5-methoxyindole)-propionic, β-3-(2-carboxy-6-methoxyindole)-propionic, and β-3-(6-methoxyindole)-propionic acids while the related β-3-(2-carboxy-7-methoxyindole)-propionic and β-3-(7-methoxyindole)-propionic acids were made by Manske (80). Jackson and Manske (53) synthesized γ-3-indolebutyric acid and also obtained a small amount of γ-3-indolebutylmalonic acid. Manske and Leitch (81) obtained Δ-3-indolevaleric, Δ-3-(2-carboxyindole)-valeric and β-3-(5-methylindole)-propionic acids. Tanaka (101) prepared 3-(2-carboxyindole)-acetic acid which he decarboxylated to 3-indoleacetic acid in low yield.

Kögl and Kostermans (77) prepared 3-(2-ethylindole)-acetic, 3-(5-methylindole)-acetic, and 3-(2,5-dimethylindole)-acetic acids by the action of alcoholic sulfuric acid on the proper hydrazones.

King and L'Ecuyer (63) prepared 3-(2-carboxyindole)-acetic acid by the Japp-Klingemann-Fischer procedures. Attempts to decarboxylate to 3-indoleacetic acid resulted in very low yields. The related 3-(1-methylindole)-acetic acid was obtained in satisfactory yield by decarboxylation of 3-(1-methyl-2-carboxyindole)-acetic acid prepared by cyclizing the methylphenylhydrazone of α-ketoglutaric acid.

Tanaka (102) also prepared 3-indoleacetic acid by the zinc chloride cyclization of the phenylhydrazone of β-formylpropionic acid.

Other methods besides the Fischer synthesis have been

used to prepare 3-indoleacetic acid and other indole derivatives. Piccinini (89) introduced an acetic acid group into a substituted indole by the use of ethyl diazoacetate and obtained 3-(1-methylindole)-acetic and 3-(2-methylindole)-acetic acids. Jackson and Manske (54) modified this method and prepared 3-indoleacetic, 1,3-indolediacetic, and 3-indolesuccinic acid.

Oddo (86) obtained 1-(3-methylindole)-carboxylic, 2-(3-methylindole)-carboxylic, and 3-(2-methylindole)-carboxylic acids by treating 3- and 2-methylindolemagnesium iodides with carbon dioxide. A similar method was used by Majima and Hoshino (79) for the preparation of 3-indoleacetic acid and β-3-indolepropionic acid. They condensed 3-indolemagnesium iodide with chloroacetonitrile and β-chloropropiononitrile.

Indolepyruvic acid was synthesized by Granacher, Gero and Schnelling (32) from 3-indolalrhodanine by treating it with aqueous potassium and ammonium hydroxide.

In 1941, several patents (6, 22, 23) for preparing indole derivatives appeared in the literature. Indoles that are unsubstituted in the 3- position were treated with formaldehyde and hydrogen cyanide or one of its salts. The nitrile obtained was hydrolyzed to the 3-indoleacetic acid. The preparation of 3-indoleacetic, 3-(2-methylindole)-acetic, 3-(2-phenylindole)-acetic and 3-(2,5-dimethylindole)-acetic acids were cited as examples.

Preparation of β-Formylpropionic Acid

Perkin and Sprankling (88) prepared β-formylpropionic acid by condensing bromoacetal with ethyl sodiomalonate. The condensation product was hydrolyzed with water, and an oil was obtained which was characterized.

Ungern-Sternburg (108) prepared what was supposed to be β-formylpropionic acid from aconic acid, but the product was crystalline and melted at 147°.

Harries and Ahlefeld (43) synthesized the substance from allylacetic acid ozonide and obtained an oil which turned to a solid upon standing. Heating and distilling the solid regenerated the oil. Harries and Himmelman (44) by molecular weight determination found that the solid was a dimer while the oil was the simple half-aldehyde.

Ellinger (19) converted itaconic acid to aconic acid which was transformed by the method of Ungern-Sternburg to β-formylpropionic acid.

This half-aldehyde was also prepared by Wislicenus, Boklen and Reuth (120) by condensing ethyl formate with diethyl succinate. The diethyl formylsuccinate was decomposed by heating with water in a sealed tube. This procedure was modified by Carrière (13) who decomposed formyl succinic acid by boiling with aqueous oxalic acid. The product obtained was a trimer, which when distilled yielded β-formylpropionic acid and a residue which seemed to be composed of two units

of the aldo-acid minus one molecule of water.

Langheld (78) oxidized glutamic acid with sodium hypochlorite and obtained β-formylpropionic acid. Dakin (16) used Chloramine-T as the oxidizing agent.

The acetal of β-formylpropionic acid, β, β-diethoxypropionate, was prepared from alcoholic sodium ethoxide and ethylpropionate by Ingold (52).

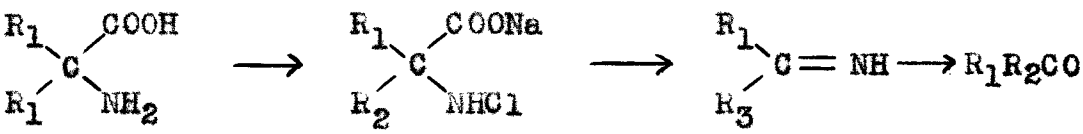
Shchukina and Preobrazhenskii (95) obtained the ethyl ester of β-formylpropionic acid by boiling the condensation product of ethyl α-formyl- γ, γ-diethoxybutyrate and ethyl α-bromobutyrate with hydrochloric acid.

Ethyl β-formylpropionate was prepared by Stoll and Bolle (99) who ozonized ethyl allylmalonate and decomposed the ozonide by catalytic hydrogenation.

Jackson and Manske (54) obtained β-formylpropionic acid by hydrolyzing β-cyanopropionacetal.

THEORETICAL

A survey of the literature showed that the method of Langheld (78) for converting amino acids to aldehydes held considerable promise as a means of converting amino acids into commercially useful intermediates. Glutamic acid, for example, was converted into β-formylpropionic acid by the action of sodium hypochlorite. This reaction was a general one, as Langheld showed by the conversion of thirteen different amino acids. He proposed the following scheme for the course of the reaction:



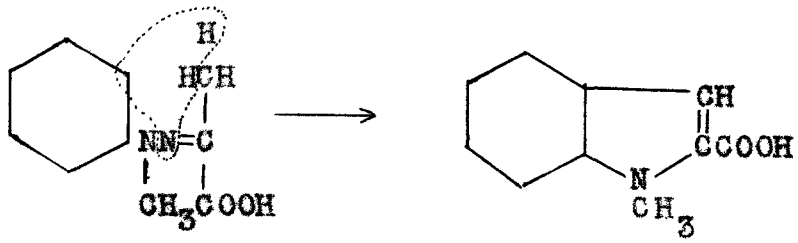
The formation of the chloroamino compound, which was comparable to the first step in the Hoffman reaction, was shown by the isolation of the mono- and dichloroaminoleucines. They were white crystalline solids that decomposed readily. This first reaction proceeded readily in the cold, while the decomposition of the chloramino compound, through the hypothetical imine, was rapidly accelerated by heat. Langheld considered it important to prevent the simultaneous presence of aldehyde and chloroamino acid, since the latter is an oxidizing agent. He accomplished the decomposition by allow-

ing the cold chloroamino acid to fall into a stream of steam. A 92 per cent yield of β-formylpropionic acid was obtained as the p-nitrophenylhydrazone. The reported yield was based on the crude hydrazone which melted at 158° while the correct melting point has been established at 185° (106). The elaborate steam distillation apparatus and the dilute solutions used limit the amounts of materials that could be conveniently prepared by this method.

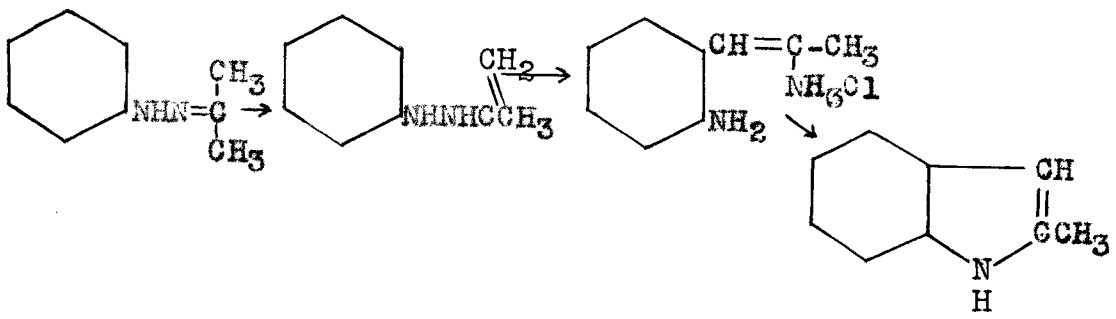
Dakin (16) investigated the reaction further and used Chloramin-T as the oxidant in place of sodium hypochlorite. With equimolar quantities, he was able to obtain an 88 per cent yield of β-formylpropionic acid by simply heating the reaction mixture to 50° to decompose the chloroamino acid. An excess of Chloramine-T, however, formed β-cyanopropionic acid. The amount of glutamic acid that Dakin converted to the half aldehyde was 0.01 mole. A larger amount, 0.037 mole, was also converted, but no quantitative data were reported.

One of the questions that this investigation attempted to answer was whether larger quantities of β-formylpropionic acid could be prepared by modifying the method without lowering the yield too much. A possible application of β-formylpropionic acid would be in the Fischer indole synthesis (27) in which its phenylhydrazone, when cyclized, would yield 3-indoleacetic acid.

There has been considerable disagreement over the mechanism of this synthesis whereby an arylhydrazone is converted to an indole by the elimination of ammonia. Fischer (26) stated that the nitrogen attached to the carbonyl carbon was eliminated together with a hydrogen from the benzene ring plus two from the methylene group that was involved in closing the ring, but he offered no mechanism.



In 1918 Robinson and Robinson (92) proposed a hypothesis for the mechanism of the Fischer reaction. The arylhydrazone was believed to be rearranged to an unsaturated hydrazine by addition of the acid reagent and decomposition of the addition product. This hydrazine then underwent an ortho-benzidine type rearrangement, and finally the new ring was formed by elimination of the ammonium salt. This mechanism can be illustrated with the synthesis of 2-methylindole from the phenylhydrazone of acetone.

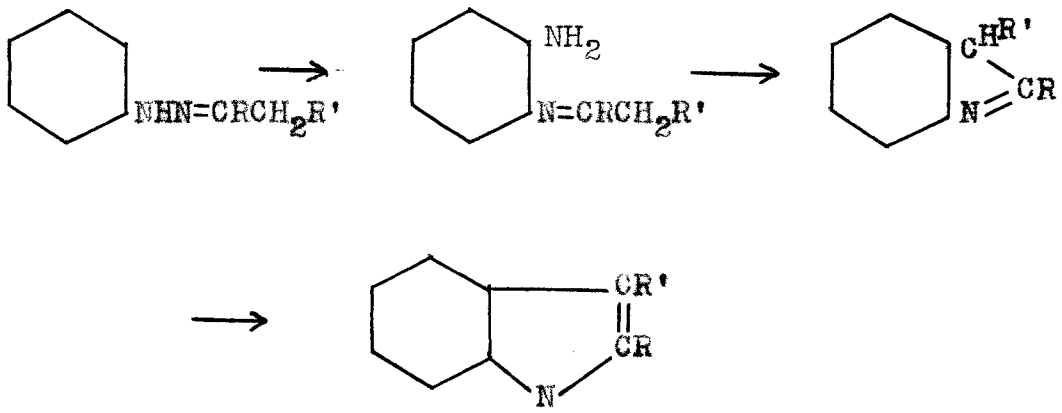


Each step in this process resulted in the formation of a substance more basic than the last until the reaction was stopped by the formation of the aromatic type ring. This type of reaction would be favored by the required acidic reagent.

If this were the correct path, the aldehydes and ketones which enolize readily should have been more easily converted to indoles than those that did not enolize. That this was so was proved by Fischer and Schmitt (28) who found that phenylacetaldehydephenylhydrazone was easily converted to 3-phenylindole while acetophenonephenylhydrazone required heating with zinc chloride at 180° (24). Semmler (94) had observed that phenylacetaldehyde was converted into the acetate of the enolic modification by boiling with acetic anhydride whereas acetophenone was not. Unsubstituted indole itself has never been prepared from the phenylhydrazone of acetaldehyde which enolizes very little (97). In general, indole formation from phenylhydrazones of aldehydes and ketones that have a negative group in the position beta to the carbonyl appeared to be favored (66).

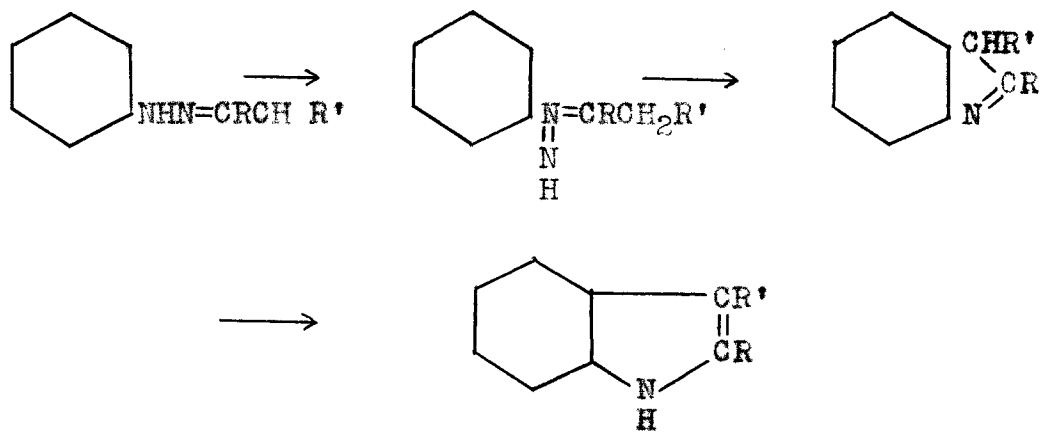
In 1922 Hollins (50) reviewed the different hypotheses in existence and gave arguments against all of them. He objected to Robinson's hypothesis because of the absence of any para isomerization products and the fact that no increase in yield was obtained when p-tolylhydrazones were used.

Cohn (15), without any experimental evidence, proposed an orthosemidine transformation:



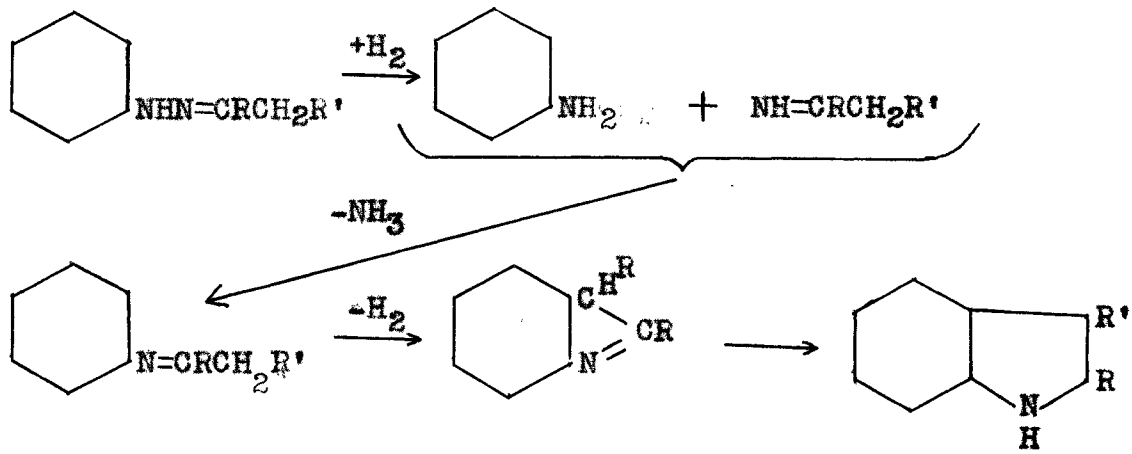
This mechanism did not explain the formation of N-substituted indoles and involved an alteration in the orientation of substituents in the benzene ring.

Still another theory was put forth by Bamberger and Landau (3) who proposed the following scheme:

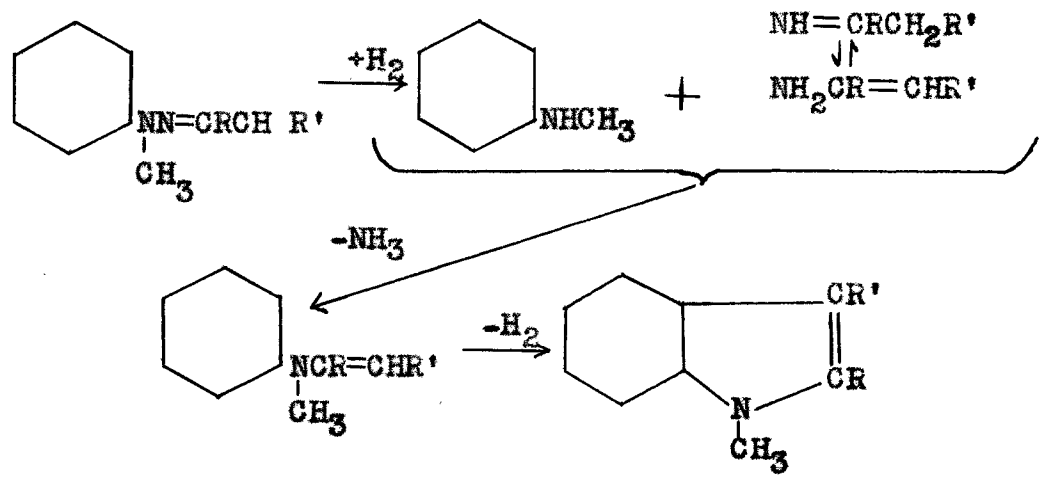


They did not explain the method of elimination of ammonia, nor did they explain the formation of N-methylindoles from methylphenylhydrazones.

The discovery that acetophenone anil may be oxidized to phenylindole by phenylhydrazine prompted Reddelien (91) to put forward the following scheme:



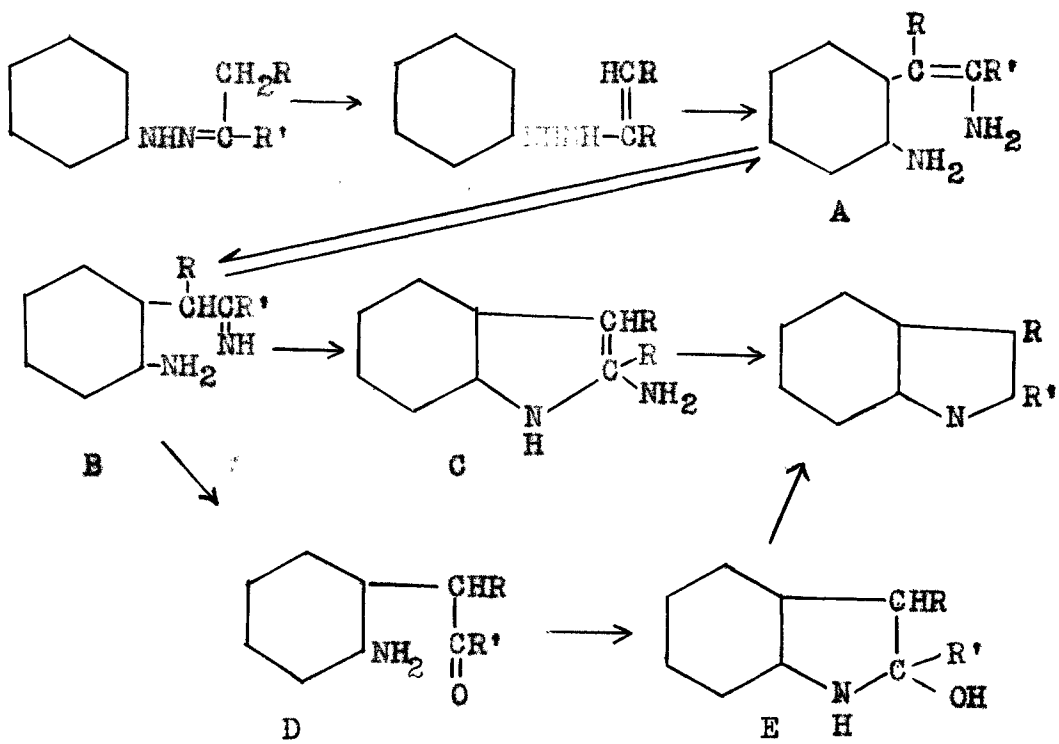
To account for the formation of N-methyl indoles, Hollins (50) modified this mechanism by suggesting that the imine formed condenses with the aryl amine in a tautomeric form.



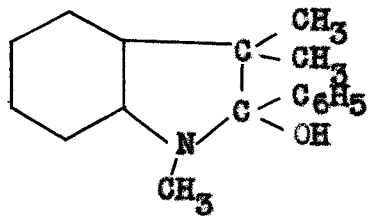
Robinson and Robinson (93), however, found that the indole synthesis proceeded normally in the presence of foreign aromatic amines which would not have been true if the

mechanism of either Reddelien or Hollins were correct. They found several other faults in the Reddelien-Hollins hypothesis: It offered no explanation for an acidic reagent, it failed to take into account the extreme sensitiveness of ketone imines and anils to hydrolysis by acids, it required fission of the hydrazone and recombination of the parts, and it involved compensating oxidation and reduction with intermediate condensation.

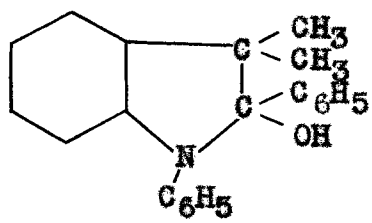
In 1943 Allen and Wilson (1), using N^{15} as a tracer element, definitely proved that the nitrogen atom in the phenylhydrazone that was eliminated was the one furthest from the benzene ring. They supported the Robinsons' view as far as the elimination of the ammonia, for which they preferred an addition-elimination mechanism:



Imide B is tautomeric with A and the existence of either is possible in the synthesis. The addition could proceed directly by path B-C or hydrolysis could occur with the formation of D which would then give the addition product E. The latter mechanism seemed to be favored by Jenisch's report (56) that the phenylhydrazone of isopropyl phenyl ketone gave



and the report of Neber et al. (83) who obtained



from the diphenylhydrazone of phenyl isopropyl ketone.

Another objective of the present research was to attempt to synthesize some 3-indoleacetic acids with groups substituted in the benzene part of the indole ring which had been shown to increase the plant growth activity of other compounds. Zimmerman and Hitchcock (125) had made the interesting observation that the order of increasing activity for the series phenoxyacetic, o-chlorophenoxyacetic, p-chlorophenoxyacetic, and 2,4-dichlorophenoxyacetic acids was 1, 20, 80 and 650.

A search of the literature revealed no such derivatives of 3-indoleacetic acid had been made. However, Borsche (8) had prepared the o-, p- and m-nitrophenylhydrazones of cyclohexanone and cyclized them to the corresponding 6, 7, 8, 9-tetrahydrocarbazoles with dilute sulfuric acid. Bauer and Strauss (7) had cyclized the o-, m- and p-nitrophenylhydrazones of methyl ethyl ketone to 7-nitro-2,3-dimethyl-, (4 or 6)-nitro-2,3-dimethyl-, and 5-nitro-2,3-dimethylindoles, but when they attempted to obtain the corresponding derivatives from the hydrazones of propionaldehyde they obtained propylidene dinitroskatoles. They also attempted to cyclize the p-nitrophenylhydrazone of levulinic acid with zinc chloride and hydrochloric acid, but were unsuccessful.

Hughes, Lions and Ritchie (51) prepared several 2-(3-alkyl-7-nitroindole)-carboxylic acids from the esters of the o-nitrophenylhydrazones of the α-keto acids from pyruvic to α-ketocaproic acids. They found that no one of the eight methods of cyclization was successful for all the derivatives prepared; a method that worked well for one hydrazone was sometimes useless for the preparation of a second. Evidently some of the yields were very low because they were not reported in all instances.

Along with the attempted syntheses of substituted 3-indoleacetic acids, the preparation of the analogous 3-(2-methylindole)-acetic acids was made since the parent com-

pound was itself about 0.5% as active as indoleacetic acid. These were obtained from the substituted phenylhydrazones of levulinic acid, β-acetyl propionic acid, a large supply of which was conveniently available.

EXPERIMENTAL

Preparation of β-Formylpropionic Acid

Langheld's method

In accordance with Langheld's procedure (78), 1.47 g. (0.01 mole) of glutamic acid[1], m.p. 199-200° (dec.), in 50 ml. of water containing 0.4 g. sodium hydroxide was treated with 10 ml. of 1 M sodium hypochlorite solution prepared by the method of Raschig (90). This solution was slowly dropped into a stream of steam in the apparatus described by Langheld. The non-volatile portion of the decomposed solution contained all the β-formylpropionic acid, which was converted to the p-nitrophenylhydrazone. The yield was 0.52 g. (87%) of crude product, m.p. 159-169°. After several recrystallizations from ethanol-water, the m.p. was 185-186° (cor.). Langheld found a melting point of 175°.

Dakin's method

Using Dakin's (16) modification, 1.0 g. (0.068 mole) of glutamic acid dissolved in 100 ml. of 0.1 M sodium hydroxide

[1] All glutamic acid used in the experiments was prepared from commercial glutamic acid by the method of King (62).

was reacted with 1.98 g. of solid Chloramine-T. The solution was heated to 50°, and then cooled after the reaction had taken place as shown by a negative test with potassium iodide and acetic acid. The p-toluenesulfonamide which had precipitated out during the reaction was filtered off and 1.04 g. (0.068 mole) of p-nitrophenylhydrazine was added to the β-formylpropionic acid solution. The precipitated hydrazone was filtered and washed with water; 1.08 g. (66%) was obtained. The melting point after several recrystallizations from water-ethanol was 185-186° (cor.). Dakin reported 185-187°.

Modified methods

Since both Langheld's and Dakin's methods involved small quantities, the oxidation of glutamic acid by active chlorine compounds was studied further in an attempt to determine what effects larger quantities of reactants and different methods of decomposition of the N-chloro-α-aminoglutaric acid would have on the yield of β-formylpropionic acid.

Decomposition by boiling. (I) To 1.00 g. (0.068 mole) of glutamic acid dissolved in 10 ml. of 1 M sodium hydroxide and diluted with 100 ml. of water, 6.8 ml. of 1 M sodium hypochlorite solution was added. The N-chloro-α-aminoglutaric acid was decomposed by bringing the solution to

boiling. A solution of 1.04 g. (0.068 mole) of p-nitrophenylhydrazine in 25 ml. of ethanol-acetic acid (1:1) was added. The mixture was heated on the steam bath for 10 minutes; 100 ml. of water was added, and the crystals which collected after the solution was cooled were filtered off. The yield was 0.96 g. (60%).

(II) A 1.00 g. sample of glutamic acid was treated as in (I) above except the solution was acidified with acetic acid before boiling to decompose the chloroamino compound. The yield was 0.93 g. (58%).

(III) Another 1.00 g. sample of glutamic acid was converted to the chloroamino compound as in (I), but the solution was boiled for 15 minutes before the p-nitrophenylhydrazine was added; yield 0.85 g. (53%).

(IV) Another experiment was performed as in (III) except the solution was acidified before boiling 15 minutes; yield 0.87 g. (54%).

(V) This experiment was performed in order to see what effect a higher concentration of reactants would have on the yield. A 14.7 g. (0.1 mole) portion of glutamic acid in 200 ml. solution was reacted with sodium hypochlorite and brought to boiling. The β-formylpropionic acid was collected as the phenylhydrazone; 5.1 g. (27%).

(VI) A 29.4 g. (0.2 mole) quantity of glutamic acid in 200 ml. was treated as in (V). The yield was 7.5 g. (19%).

Decomposition at lower temperatures. (I) To a 14.7 g. (0.1 mole) quantity of glutamic acid in 200 ml. of 0.5 M sodium hydroxide, 100 ml. of 1 M sodium hypochlorite was added and the resulting solution was heated for 15 minutes on a steam bath, at which point a negative active chlorine test was obtained. An equivalent amount of p-nitrophenylhydrazine in hot dilute acetic acid (1:1) was added. The flask was heated for a few minutes on the steam bath and then cooled; yield 8.7 g. (37%).

(II) A sample of glutamic acid weighing 7.35 g. (0.05 mole) was dissolved in 100 ml. of 0.5 M sodium hydroxide and 4.5 g. (0.05 mole) of solid chloramine-T was added. The temperature was raised to 50° and the reaction was allowed to proceed to completion as shown by a negative chlorine test. The precipitated p-toluenesulfonamide was filtered off and discarded; the filtrate containing the β-formylpropionic acid was treated with a hot solution of 9.5 g. (0.05 mole) of p-nitrophenylhydrazine and 4.1 g. (0.05 mole) of sodium acetate in 25 ml. of dilute acetic acid (1:1). The yield of hydrazone was 6.4 g. (53%).

(III) A solution of 14.7 g. (0.1 mole) glutamic acid in 400 ml. of water containing 4 g. of sodium hydroxide in a three-necked flask fitted with a thermometer, stirrer, and dropping funnel, was reacted with 100 ml. of 1 M sodium hypochlorite. The flask was heated to 50° and held at this tem-

perature while dilute acetic acid was added slowly to acidity (litmus). An evolution of carbon dioxide took place upon the addition of the acid. When the reaction was complete (negative starch-iodide test) an equivalent quantity of p-nitrophenylhydrazine in hot dilute acetic acid was added; yield 15.0 g. (63%).

Attempted isolation of β-formylpropionic acid

(I) A 14.7 g. (0.1 mole) portion of glutamic acid was oxidized with sodium hypochlorite. The solution was acidified with hydrochloric acid and concentrated under reduced pressure until crystals settled out. These were probably inorganic since they did not melt upon strong ignition. The filtrate was distilled at 3-4 mm.; a small fraction was collected at 78° which solidified upon standing. Recrystallization from ethanol-ether eventually gave a solid which did not melt under 225°. The solid volatilized upon strong heating, and was probably a polymer of β-formylpropionic acid.

(II) The solution obtained from the oxidation of 29.4 g. (0.2 mole) of glutamic acid with Chloramine-T was made basic with sodium bicarbonate and extracted with ether in a liquid-liquid extractor to remove p-toluenesulfonamide which other experiments had indicated was not completely removed by filtration. Evaporation of the ether solution gave a crystalline material which proved to be p-toluenesulfonamide, m.p. 137-137.5°, mixed m.p. with authentic p-toluenesulfonamide 137-

137.5°. The water phase, when acidified, gave strong Fehling and Schiff tests. Evaporation on the steam bath yielded a brown resinous material which was not investigated further.

The attempted isolation was not carried further since the pure compound itself is unnecessary in subsequent work. The dilute aqueous solution obtained upon oxidation can be utilized.

Preparation of 3-Indoleacetic Acid

(I) The β-formylpropionic acid prepared by oxidizing 0.1 mole of glutamic acid with sodium hypochlorite at 50° was reacted with phenylhydrazine. The solution was concentrated under reduced pressure to give a red oil mixed with a precipitate. The solid was separated from the precipitate, which proved to be inorganic, by extraction with absolute ethanol. Evaporation of the ethanol left 5.0 g. of a red oil which would not crystallize. Jackson and Manske (54) also report the phenylhydrazone as a red oil.

A 4.0 g. portion of this oil was treated in accordance with the procedure of Ellinger (19). The oil was refluxed with 100 ml. of 10% sulfuric acid for four hours, and then the solution was diluted with 300 ml. of water which precipitated a brown oil that was separated by extracting the aqueous solution four times with 150 ml. portions of ether. The extract was dried with "Drierite" and the ether distilled

off. The residue was saponified under reflux with 20 ml. of 10% alcoholic potassium hydroxide for one hour. The mixture was diluted to 100 ml. and acidified with sulfuric acid. A brown smeary precipitate was obtained which according to Ellinger should have consisted of only a small part of 3-indoleacetic acid. The filtrate was extracted with ether until a negative indole test was obtained. The precipitate was dissolved in dilute sodium hydroxide and the solution was acidified with sulfuric acid and filtered. The filtrate was extracted with ether. The combined ether extracts were dried and the ether was distilled off; yield 0.4 g. (11%). The residue was dissolved in hot benzene and treated with charcoal. The crystalline plates obtained, 0.22 g. (6%), melted at 166-167° (cor.) with evolution of a gas.

(II) A 0.2 mole quantity of glutamic acid was oxidized with solid Chloramine-T at 50°. The precipitated p-toluenesulfonamide was filtered off after cooling the solution. The filtrate was reacted with 0.2 mole of phenylhydrazine and the solution was evaporated to dryness under reduced pressure. The red oily phenylhydrazone was extracted with 150 ml. of absolute ethanol. Forty ml. of sulfuric acid was added and the solution was refluxed for five hours. The alcoholic solution was poured into 500 ml. of water, and the precipitated oil was extracted with ether, which was washed with sodium bicarbonate and water. The ether was distilled

off after drying the extract over calcium sulfate. Following the directions of Jackson and Manske (54) for separating the crude ethyl 3-indoleacetate, the residue was distilled at 2 mm. and the fraction boiling above 150° was collected; 5.1 g. of crystalline material was obtained. Recrystallization of a portion of the residue from ethanol gave crystals, which when washed several times with benzene, melted at 137.5-139°. A mixed melting point with p-toluenesulfonamide showed no depression.

The remaining portion of the distillate, 3.8 g., was saponified with 40 ml. of 10% alcoholic potassium hydroxide. The solution was diluted, evaporated partially to remove the alcohol, and then acidified with hydrochloric acid. A crystalline precipitate, m.p. 137.5-139°, was obtained and was shown to be p-toluenesulfonamide. The filtrate was extracted with ether, which when evaporated, yielded a residue. Recrystallization from benzene gave 0.5 g. of crystalline plates, m.p. 164-165°.

(III) In the preparation of 3-indoleacetic acid from β-formylpropionic acid phenylhydrazone, prepared by oxidation of glutamic acid with Chloramine-T, p-toluenesulfonamide was carried along as a contaminant. An attempt was made, therefore, to purify the red oily phenylhydrazone by vacuum distillation. A 0.05 mole portion of glutamic acid was oxidized with Chloramine-T and converted into the phenylhydra-

zone in the usual manner. The β-formylpropionic acid phenylhydrazone was extracted with ether; the extract was dried with "Drierite", the ether distilled, and the residue dried at 100° under reduced pressure. That the residue was not pure phenylhydrazone was shown by titration; a neutral equivalent of 400 was obtained instead of the theoretical 192.

The remainder of the residue was distilled at 2 mm. and yielded a fraction boiling between 135-151° which solidified, m.p. 85-90°. Recrystallization of the solid from water was unsuccessful.

The preparation of the hydrazone was repeated by starting with 0.10 mole of glutamic acid. Distillation at 2-3 mm. yielded a fraction of b.p. 143-146°. The oily liquid solidified upon cooling; yield 2.5 g., m.p. 91-94°. Recrystallization from water raised the melting point to 94-95° (cor.).

Anal. Calc'd. for $C_{10}H_{10}ON_2$: N, 16.1

Found: N[1], 16.02, 16.24

The compound was insoluble in cold water and cold 5% hydrochloric acid, but was soluble in hot 5% sodium hydroxide immediately or, after a long time, in cold 5% sodium hydroxide. A red solution was obtained which turned yellow upon acidification.

The behavior and analysis for the compound indicated

[1]All nitrogen determinations were carried out by the micro-Dumas method.

that it was the anhydride of the phenylhydrazone of β-formylpropionic acid. Fischer (24) had obtained the analagous compound from the phenylhydrazone of levulinic acid by heating at an elevated temperature.

(IV) To 29.4 g. (0.2 mole) of glutamic acid in 400 ml. of 0.5 M sodium hydroxide solution, 200 ml. of 1 M sodium hypochlorite solution was added. The solution was heated to 50° and dilute hydrochloric acid was added to neutrality. The reaction was kept at 50° until a negative starch-iodide test was obtained, at which point 22.0 g. (0.2 mole) of phenylhydrazine in dilute acetic acid was added. The solution was heated on the steam bath for a few minutes and allowed to stand for one hour. The red oil which had precipitated out was extracted with ether. The extract was dried with "Drierite" and the ether distilled off; the final solvent was removed under reduced pressure at 60°. The weight of the red, oily phenylhydrazone was 25.6 g. (67%).

The phenylhydrazone was refluxed with 40 g. of sulfuric acid and 200 ml. of absolute ethanol for five hours, and then diluted with a liter of water. The aqueous solution was extracted with ether until a negative indole test was obtained with p-dimethylaminobenzaldehyde in concentrated sulfuric acid. The extract was dried with "Drierite" and the ether was distilled off.

The residue obtained was saponified with 80 ml. of 10%

alcoholic potassium hydroxide under reflux for one hour. The alcoholic solution was diluted to 500 ml. and filtered to remove a dark precipitate. The filtrate was extracted with ether until a negative indole test was obtained. The precipitate was dissolved in dilute potassium hydroxide and the solution was acidified and filtered again. The filtrate was extracted with ether. The combined ether extracts were dried with "Drierite" and the ether was distilled off; yield 11.0 g. of crude indoleacetic acid; this corresponds to a 30% yield based on glutamic acid. The residue was recrystallized from benzene after reprecipitation from potassium hydroxide and ether extraction; yield 8.0 g. (22%), m.p. 164-166° (cor.).

Attempted Preparation of 3-(5-Nitroindole)-acetic Acid

A solution of 4.74 g. (0.02 ml.) of p-nitrophenylhydrazone of β-formylpropionic acid in 100 ml. of 15% ethanolic sulfuric acid was refluxed for four hours. The solution was diluted with 500 ml. of water, and the bright yellow suspension that was formed was extracted with ether. The extract was dried with "Drierite" and the ether was distilled off. The residue was refluxed with 50 ml. of 10% alcoholic potassium hydroxide for two hours. The solution turned from a deep brown to a brilliant purple upon the addition of the alkali. The basic solution was diluted and acidified. A

smeary precipitate formed which was filtered off. The filtrate was extracted with ether. The precipitate was dissolved in dilute potassium hydroxide, and the alkaline solution was acidified. After filtration, the filtrate was extracted with ether. The combined ether extracts were dried with "Drierite" and the ether was distilled off. The small amount of brown oily material was dissolved in benzene but did not crystallize on slow evaporation of the solvent. Since the amount of material obtained was very small, the experiment was not carried further.

Attempted Preparation of 3-(7-Nitroindole)-acetic Acid

β-Formylpropionic acid o-nitrophenylhydrazone

A solution of 14.7 g. (0.1 mole) glutamic acid in 400 ml. of water containing 4 g. sodium hydroxide was oxidized with 100 ml. of 1 M sodium hypochlorite at 50°. The solution was acidified and kept at 50° until a negative starch-iodide test was obtained. To one-half of this solution an equivalent quantity of o-nitrophenylhydrazine, 7.7 g. (0.05 mole), was added; yield 7.4 g. (62%), m.p. 149-152°. Recrystallization from ethanol-water with "Norit" decolorization raised the melting point to 155-156° (dec.)(cor.).

Anal. Calcd. for $C_{10}H_{11}O_4N_3$: N, 17.71; neut. equiv., 237.2

Found: N, 17.44, 17.36; neut. equiv., 239.0
(End point determined with pH meter)

Attempted cyclization of β-formylpropionic acid o-nitrophenylhydrazone with sulfuric acid

One g. (0.0042 mole) of the hydrazone prepared above was dissolved in concentrated sulfuric acid. After twenty-four hours at room temperature, the solution was poured into a large amount of water. The aqueous solution was extracted with an equal volume of ether, used in several portions. The extract was dried with "Drierite" and evaporated. The residue was dissolved in benzene and allowed to crystallize; 0.5 g. (26%) of crystals, m.p. 99-102°, was obtained. Several recrystallizations from ethanol-water raised the melting point to 101.5-102° (cor.).

Anal. Calcd. for $C_{10}H_8O_4N_2$: N, 12.72

Calcd. for $C_{10}H_9O_3N_3$: N, 19.17

Found: N, 19.43, 19.24

The compound was not soluble in 5% hydrochloric acid, or immediately soluble in cold 5% sodium hydroxide, but dissolved slowly in cold, or rapidly in hot, sodium hydroxide solution to give a deep red-brown solution. The color of the solution changed to yellow upon acidification. This was the reverse of the color change observed when β-formylpropionic acid o-nitrophenylhydrazone was titrated with alkali. The compound was another of the hydrazone anhydride series.

Attempted cyclization of β-formylpropionic acid o-nitrophenylhydrazone with alcoholic hydrogen chloride

Dry hydrogen chloride was passed into a solution of 1.00 g. (0.0042 mole) of β-formylpropionic acid o-nitrophenylhydrazone in 60 ml. of absolute ethanol and the solution was refluxed for two hours. After cooling, the mixture was poured into 350 ml. of water, and the solution was extracted four times with 100 ml. of ether. The combined ether extracts were dried with "Drierite" and the ether was distilled off; yield 0.6 g. The residue was decolorized with "Norit" and recrystallized from ethanol-water to give orange-red crystals, m.p. 75-75.5° (cor.).

Another 2.00 g. of the phenylhydrazone treated identically, yielded 1.45 g. of material which melted at 75-75.5° (cor.) after recrystallization from ethanol-water. The melting point was not raised by recrystallization from benzene.

Anal. Calcd. for $C_{12}H_{17}O_8N_3$: N, 15.84

Found: N, 15.62, 15.71

A portion (0.5 g.) of the compound was saponified with alcoholic potassium hydroxide. The solution was diluted, acidified, and extracted with ether. The extract yielded a small amount of dark oily residue when the ether was evaporated. The residue was taken up in ether and extracted with sodium bicarbonate solution. The bicarbonate fraction was

acidified and extracted with ether, which when dried and evaporated, yielded no residue.

Attempted cyclization of ethyl β-formylpropionate o-nitrophenylhydrazone

Since the compound appeared to be ethyl β-formylpropionate o-nitrophenylhydrazone, an attempt was made to cyclize the latter with zinc chloride. The method used by Fischer (24) for ethyl levulinate phenylhydrazone was followed. Four-tenths g. of the ester was heated with 2 g. of anhydrous zinc chloride[1] for one hour in an oil bath held at 140°. The melt was dissolved in dilute hydrochloric acid and extracted many times with ether. The combined extracts were dried with "Drierite" and the ether was distilled off. The residue was refluxed for one hour with 10% methanolic potassium hydroxide. The methanol was removed by distillation at reduced pressure after 50 ml. of water had been added. The aqueous solution was extracted with ether to remove non-acid material. The basic solution was acidified and extracted with four 50 ml. portions of ether. After drying, the ether was distilled, leaving a residue of about 7 mg.

[1]All the zinc chloride used in this investigation was prepared by dissolving zinc chloride in concentrated hydrochloric acid and evaporating in an atmosphere of hydrogen chloride (29).

Preparation of 3-(2-Methyl-7-nitroindole)-acetic Acid

Levulinic acid o-nitrophenylhydrazone

A hot solution of 7.65 g. (0.05 mole) of o-nitrophenylhydrazine in dilute acetic acid was added to 5.8 g. (0.05 mole) of levulinic acid in 200 ml. of hot water. The red-orange oil which precipitated crystallized upon cooling; yield 10.2 g. (80%). The material was recrystallized from water-ethanol, m.p. 149-150°. Two more recrystallizations from the same solvent brought the melting point up to 150-150.5° (cor.).

Anal. Calcd. for $C_{11}H_{13}O_4N_3$: N, 16.73

Found: N, 16.92, 16.28

Ethyl levulinate

The compound was prepared according to the directions of Grote et al. (33). Dry hydrogen chloride was rapidly passed into a solution of 116.0 g. (1 mole) of levulinic acid in 250 ml. of absolute ethanol. The mixture was refluxed for one hour and the alcohol was fractionated off. The residue distilled at 205° and yielded 120.0 g. (83%).

Ethyl levulinate o-nitrophenylhydrazone

A hot solution, consisting of 15.3 g. (0.1 mole) of o-nitrophenylhydrazine, 9.0 g. (0.11 mole) of sodium acetate,

10 ml. of acetic acid, 100 ml. of ethanol, and 200 ml. of water, was added to 12.8 g. (.11 mole) of ethyl levulinate in 200 ml. of water. An orange oily layer appeared immediately and crystallized upon cooling in the refrigerator; yield 18.5 g. (66.5%), m.p. 59.5-60° (cor.).

Attempted cyclization of levulinic acid o-nitrophenylhydrazone with hydrogen chloride

Dry hydrogen chloride was bubbled rapidly into a solution of 1.00 g. (0.0040 mole) of the acid hydrazone in 60 ml. of absolute ethanol, and the solution was refluxed for two hours. The preparation was diluted with 200 ml. of water and extracted with four 50 ml. portions of ether. The combined ether extracts were washed with sodium bicarbonate and water. After drying with "Drierite" the ether was distilled off and the residue was recrystallized from alcohol; yield 0.88 g., m.p. 57.5-58.5° (cor.). Recrystallization from water-alcohol yielded orange crystals, m.p. 58.5-59° (cor.). A mixed melting point with ethyl levulinate o-nitrophenylhydrazone, prepared from ethyl levulinate and o-nitrophenylhydrazine, showed no depression.

Anal. Calcd. for $C_{13}H_{17}O_4N_3$: N, 15.05

Found: N, 14.89

Attempted cyclization of levulinic acid o-nitrophenylhydrazone with sulfuric acid

Two g. (0.0080 mole) of the acid hydrazone was dissolved in concentrated sulfuric acid and kept at room temperature for twenty-four hours. Dilution of the acid solution and extraction with ether yielded an oil which when recrystallized from alcohol proved to be the starting material, m.p. 57.5-58.5° (cor.); a mixed melting point with levulinic acid o-nitrophenylhydrazone showed no depression.

Cyclization of ethyl levulinate o-nitrophenylhydrazone with zinc chloride

(I) A preliminary experiment was run by heating 0.5 g. of ester hydrazone mixed with 2.5 g. of anhydrous zinc chloride, for one hour at 140°. The melt was dissolved in 50 ml. of 1 M hydrochloric acid, and the solution obtained was extracted four times with 50 ml. portions of ether. The ether extracts were dried with "Drierite", and the ether removed by distillation. The residue was refluxed for forty-five minutes with methanolic potassium hydroxide, water was added; and most of the methanol was removed by distillation under reduced pressure. The solution was diluted, acidified, and then extracted with ether. The extract was dried and the ether distilled off. The residue was taken up in a small amount of hot acetic acid. On cooling, no crystals appeared, so

water was slowly added; a small amount of brown flocculent precipitate appeared and was filtered off. The amount was very small and could not be scraped from the filter paper. The filtrate was treated with "Norit" and the solvent was removed in a vacuum dessicator. About 20 mg. of a yellow waxy crystalline material was obtained. Titration of the residue indicated a neutral equivalent of 260; the calculated value for 3-(2-methyl-7-nitroindole)-acetic acid is 234.2.

(II) A mixture of 3.0 g. (0.011 mole) of the ester and 15.0 g. of anhydrous zinc chloride was heated in an oil bath at 135-145° for three hours. The temperature accidentally reached 170° for a very short time. The melt was dissolved in 1 M hydrochloric acid. Some charring had taken place and the black precipitate was filtered off. The filtrate was extracted with 50 ml. of ether four times. The precipitate was leached with ether. The combined extracts were dried and the ether distilled off. The residue was refluxed with 10% methanolic potassium hydroxide for one hour, and then the methanol was distilled off after adding an equal amount of water. The solution was extracted with ether, which was discarded, and then acidified. The acid solution was extracted four times with ether. Removal of the ether gave 0.28 g. (11%) of solid material which gradually darkened above 200°. Several recrystallizations from ethanol-water gave a material which started to darken at about 255° and decomposed at about 264° (cor.)

Cyclization of ethyl levulinate o-nitrophenylhydrazone with zinc chloride in hydrochloric acid

To a saturated solution of zinc chloride in concentrated hydrochloric acid, 2.0 g. (0.0071 mole) of the ester hydrazone was added and the solution was refluxed for one hour. The solution was extracted with ether four times, and the combined extract was dried. The ether was distilled off and the residue refluxed with alcoholic sodium hydroxide. The alcohol was removed by distillation after the addition of water. The alkaline solution was then extracted with ether which was discarded. The aqueous solution was acidified and extracted three times with portions of ether. The combined ether extracts were dried and the ether was removed by distillation; yield 0.47 g. (28%), the material decomposed about 245°. Recrystallization by dissolving in acetic acid and adding water gave a material that started to darken about 250° and melted with decomposition about 265°(cor.). The material was soluble in ethanol, acetic acid, and sodium bicarbonate solution but insoluble in water.

Anal. Calcd. for $C_{10}H_{10}O_4N_2$: N, 11.96

Found: N, 11.91, 11.82

Attempted cyclization of ethyl levulinate o-nitrophenylhydrazone by other methods

Several other conditions were employed in order to bring about ring closure but none were successful. They were:

1) Zinc chloride at 170° for two hours
2) Zinc chloride at 120° for two hours
3) Zinc chloride in boiling xylene for two hours
4) Aluminum chloride in Skelly B

Preparation of 3-(2-Methyl-7-chloroindole)-acetic Acid

o-Chlorophenylhydrazine hydrochloride

The directions of Hewitt (46) were modified slightly. Forty-five grams (0.353 mole) of o-chloroaniline dissolved in 675 g. of concentrated hydrochloric acid and well cooled in an ice salt-mixture was diazotized by slowly adding a solution of 26 g. of sodium nitrite in 180 g. of water until the reaction was complete (starch-iodide test). The mixture was kept well stirred mechanically and the temperature was kept below 5°. The solution was filtered rapidly, and 152 g. of stannous chloride dissolved in an equal weight of concentrated hydrochloric acid was added. Long crystals of o-chlorophenylhydrazine hydrochloride appeared after one half hour. The product was collected, dissolved in water, and treated with hydrogen sulfide to remove tin. After the

tin sulfide was removed by filtration, the hydrochloride was reprecipitated by the addition of concentrated hydrochloric acid to the filtrate. The material was filtered and washed with concentrated acid. The yield was 50.1 g. (71%), m.p. 189-190° (dec.). Hewitt gave 190°.

Ethyl levulinate o-chlorophenylhydrazone

A hot solution of o-chlorophenylhydrazine, prepared by dissolving 17.9 g. (0.1 mole) of o-chlorophenylhydrazine hydrochloride, 8.5 g. (0.104 mole) of sodium acetate, and 25 g. of acetic acid in 200 ml. of water, was slowly added to 14.4 g. (0.1 mole) of ethyl levulinate in 300 ml. of hot water. A light yellow oil immediately precipitated out, and solidified after cooling. The precipitate was filtered and washed with water; yield 25.5 g. (95%). Recrystallization from ethanol-water gave a white crystalline precipitate, m.p. 58.5-59.5° (cor.). The material was unstable in the air and formed a dark brown oil after short exposure. Kögl and Kostermans (77) reported the same behavior for levulinic acid p-tolylhydrazone.

Cyclization of ethyl levulinate o-chlorophenylhydrazone with zinc chloride

(I) A preliminary experiment was made using 3.0 g. (0.011 mole) of the ester hydrazone and 15 g. of zinc chlo-

ride. The mixture was heated for one hour at 100°, and the melt was dissolved in 50 ml. of 1 M hydrochloric acid. A brown oil separated out and was extracted with two 100 ml. portions of ether. The ether was dried with "Drierite" and evaporated off under reduced pressure. A brown oil remained which was refluxed for forty minutes with 25 ml. of 10% methanolic potassium hydroxide.

The methanol was distilled off under reduced pressure after adding 50 ml. of water. The aqueous solution was extracted with ether to remove non-acid organic products, and then the basic solution was acidified with dilute hydrochloric acid; the precipitated brown oil was taken up in ether. The solution was extracted two more times with 50 ml. portions of ether. The combined extracts were dried with "Drierite" and the ether was distilled off. A residue remained which was taken up in hot acetic acid and treated with "Norit". The addition of water to the acetic acid solution precipitated a crystalline product together with a gummy tar. By adjusting the concentration of acetic acid, the gum was dissolved. The crystalline material melted at 157-160°. Recrystallization of the small amount of material was unsuccessful.

(II) A 3.0 g. portion of the ester hydrazone was treated identically as in (I) to the point where the alkaline solution was acidified and extracted with ether. The ether ex-

tract was thoroughly shaken with 50 ml. of half-saturated sodium bicarbonate. The bicarbonate layer was separated and acidified. The brown oil which separated was extracted with four 50 ml. portions of ether. The combined ether extracts were dried with "Drierite" and the ether was distilled off under reduced pressure. The brown oily residue was dissolved in acetic acid, treated with "Norit", and the solution was filtered. The acetic acid was removed in a vacuum desiccator containing solid sodium hydroxide. The yield was 0.8 g. (33%) of m.p. 157-159°. The material darkened slightly at the melting point and when heated above the melting point gave off bubbles of gas. An indole test using Ehrlich's reagent (2% p-dimethylaminobenzaldehyde in ethanol-hydrochloric acid) was negative in the cold but positive upon warming. This behavior is typical for 2,3-disubstituted indoles (109). A sodium fusion and subsequent qualitative tests showed the presence of halogen and nitrogen. Two recrystallizations from benzene raised the melting point to 159-160°, and the compound gave a neutral equivalent of 231.5, (theoretical value, 223.5). Recrystallization from benzene, after treatment with "Norit" and again from benzene-petroleum ether gave tiny, slender, white needles, m.p. 164-164.5° (cor.).

Anal. Calcd. for $C_{11}H_9O_2NCl$: N, 6.27; neut. equiv., 223.5

Found: N, 6.12, 6.36; neut. equiv., 226.0, 224.6 (Phenolphthalein indicator)

(III) A 7.5 g. portion of the ester hydrazone treated as in (II) yielded 2.7 g. (43%) of the crude product.

Preparation of 3-(2-Methyl-5-chloroindole)-acetic Acid

p-Chlorophenylhydrazine hydrochloride

This compound was prepared by Hewitt's (46) procedure. A solution of p-chloroaniline dissolved in 700 g. of hydrochloric acid was diazotized by slowly adding a solution of 26 g. of sodium nitrite in 180 g. of water. The mixture was stirred vigorously with a mechanical stirrer and was kept between 0-2°. The reaction was followed with starch-iodide paper near the end of the diazotization. After the diazotization was complete the solution was rapidly filtered and 152.0 g. of stannous chloride dissolved in an equal weight of concentrated hydrochloric acid was added. A voluminous precipitate appeared immediately, was filtered off, and recrystallized twice from concentrated hydrochloric acid; yield 45.0 g. (64%), m.p. 225° (dec.)(cor.), m.p. in literature (118) was 225-230°.

p-Chlorophenylhydrazone of ethyl levulinate

A hot solution of 9.0 g. (0.050 mole) of p-chlorophenylhydrazine hydrochloride, 6.0 g. of sodium acetate, and 25.0 g. of acetic acid in 100 ml. of water was slowly added to

7.5 g. (0.052 mole) of ethyl levulinate in 150 ml. of hot water. A light brown oil separated and crystallized upon cooling. The crystalline deposit was filtered off and dried in a vacuum desiccator; the yield was 8.5 g. (63%), m.p. 104-106°. The material darkened and started to turn into an oil in the air. Recrystallization from ethanol yielded 2.0 g. of material, m.p. 104-106° (cor.)

Cyclization of p-chlorophenylhydrazone of ethyl levulinate with zinc chloride

Six g. (0.022 mole) of crude ester, m.p. 104-106°, was mixed thoroughly with 36 g. of anhydrous zinc chloride and heated in an oil bath at 125-135° for one hour. The solidified melt was dissolved in 80 ml. of 1 M hydrochloric acid with the aid of 100 ml. of ether, by shaking successively with the ether and acid solutions. After separating the ether layer, the acid layer was further extracted with ether until a negative indole test was obtained with p-dimethylaminobenzaldehyde in concentrated sulfuric acid. Three extractions with 100 ml. portions of ether were required. The combined extracts were dried and the ether was distilled off. The brown oily residue was refluxed for twenty minutes with 25 ml. of ethanol which contained 3.0 g. of potassium hydoxide.

The methanol was removed under vacuum after 50 ml. of

water had been added. The basic solution was extracted with ether, which was discarded, and then acidified with 1 M hydrochloric acid. The oily precipitate that appeared was extracted with ether until an indole test was negative. The combined ether extracts were shaken thoroughly with 100 ml. of half-saturated sodium bicarbonate solution, which was separated and carefully acidified. Again an oily precipitate appeared and was extracted with ether. The extract was dried and the ether was distilled off leaving a residue of 2.2 g. (44%) m.p. 183-186° (dec.). Recrystallization from benzene-petroleum ether yielded 1.75 g. of material, m.p. 190° (dec.) (cor.). Another recrystallization did not raise the melting point.

Anal. Calcd. for $C_{11}H_9O_2NCl$: N, 6.27; neut. equiv. 223.5

Found: N, 6.36, 6.37; neut. equiv. 221.4, 224.0 (Phenolphthalein indicator)

The acid is soluble in alcohol, ether and benzene; insoluble in water and petroleum ether.

Preparation of 3-(2-Methyl-5,7-dichloroindole)-acetic Acid

2,4-Dichlorophenylhydrazine hydrochloride

Hewitt's (46) procedure for preparing o-chlorophenylhydrazine hydrochloride was modified slightly. Dichloroaniline, 57.2 g. (0.353 mole), was dissolved in 700 g. of hydrochloric acid, cooled to -5°. and diazotized with a solution

of 26 g. of sodium nitrite dissolved in 180 ml. of water. The nitrite solution was added slowly over a period of 45 minutes with rapid mechanical stirring. The temperature was kept below 5° at all times. When the reaction was complete, as shown by the starch-iodide test, the solution was filtered rapidly and 152 g. of stannous chloride in an equal weight of hydrochloric acid was added. Crystals of the hydrochloride appeared in a few minutes and after a half hour were collected by filtration. Recrystallization from concentrated hydrochloric acid yielded 55.1 g. (73%) of the hydrochloride, m.p. 208-209° (dec.). Chattaway and Pearse (14) reported the melting point as 210° with decomposition.

2,4-Dichlorophenylhydrazone of ethyl levulinate

A hot solution of 9.3 g. (0.05 mole) of 2,4-dichlorophenylhydrazine hydrochloride, 30 g. of acetic acid, and 4.5 g. of sodium acetate in 100 ml. of water was added to a solution of 7.5 g. (0.052 mole) of ethyl levulinate in 200 ml. of water. A light brown oil separated out and crystallized upon cooling. The crystals were collected, washed with ethanol, and dried in a vacuum desiccator. The yield was 12.2 g. (81%), m.p. 74-76° (cor.) (dec.). The hydrazone decomposed in the air.

Cyclization of ethyl levulinate 2,4-dichlorophenylhydrazone with zinc chloride

Ten g. (0.033 mole) of the ester hydrazone was heated with 50 g. of anhydrous zinc chloride in an oil bath at 165-170° for an hour. The solidified melt obtained was shaken alternately with 100 ml. of ether and 100 ml. of 1 M hydrochloric acid until it was dissolved. The ether was separated and the acid solution was extracted with three 100 ml. portions of ether. No positive indole test was obtained with any of the extracts. The combined extracts were dried and the ether was distilled off under reduced pressure. The residue was refluxed with 5 g. of potassium hydroxide in 50 ml. of methanol for twenty minutes.

The methanol was removed under reduced pressure after adding 50 ml. of water. The basic solution was extracted with ether, which was discarded. The aqueous solution was acidified with dilute hydrochloric acid; an oil separated and was extracted with three 100 ml. portions of ether. The combined extracts were shaken thoroughly with 100 ml. of half-saturated sodium bicarbonate solution which was separated, carefully acidified, and extracted with three 100 ml. portions of ether. The combined extracts were dried with "Drierite", and the ether was evaporated; yield 3.2 g. (43%) of residue, m.p. 215 (dec.). Recrystallization from benzene-petroleum ether raised the melting point to 220-221° (dec.) (cor.)

Anal. Calcd. for $C_{11}H_8O_2NCl_2$: N, 5.43; neut. equiv., 258

Found: N, 5.42, 5.36; neut. equiv., 263.1, 260.2 (Phenolphthalein indicator)

Attempted Preparation of 3-(5,7-dichloroindole)-acetic Acid

β-Formylpropionic acid

A solution of 29.4 g. (0.2 mole) of glutamic acid, dissolved in 400 ml. of 0.5 M sodium hydroxide solution, was placed in a three necked flask which was fitted with a mechanical stirrer, thermometer, and dropping funnel. The solution was oxidized by slowly adding 200 ml. of 1 M sodium hypochlorite. The solution was acidified with acetic acid and warmed to 50° until a negative starch-iodide test for active chlorine was obtained.

2,4-Dichlorophenylhydrazone of *β*-formylpropionic acid

A hot solution of 26.4 g. (0.13 mole) of 2,4-dichlorophenylhydrazine hydrochloride, 15 g. of sodium acetate and 40 ml. of acetic acid in 200 ml. of water was added to the solution of *β*-formylpropionic acid prepared above. A tacky brown precipitate was obtained, filtered off, and recrystallized from alcohol. The first crop of crystalline material obtained weighed 20 g. and by concentrating the mother liquor another 10 g. was recovered; yield 30 g. (57%), m.p. 152-159°. Recrystallizations from ethanol and acetic acid raised

the m.p. to 181-182° (cor.) (dec.).

Anal. Calcd. for $C_{10}H_{10}O_2N_2Cl_2$: N, 10.73; neut. equiv., 261.1

Found: N, 10.40, 10.62; neut. equiv., 262.5, 263.1 (Phenolphthalein indicator)

Attempted cyclization of β-formylpropionic acid 2,4-dichlorophenylhydrazone with alcoholic hydrogen chloride

A solution of 17.5 g. (0.064 mole) of the hydrazone, dissolved in 100 ml. of ethanol, was refluxed and dry hydrogen chloride was passed in. After the reaction had proceeded a short time, a precipitate began to settle out. The solution was allowed to stand overnight and the crystalline deposit was filtered off. Evaporation of the ethanol yielded two more crops of crystals to give a total of 18.5 g. of the product, m.p. 212-213°. Recrystallization from alcohol raised the m.p. to 215-216° (cor.) (dec.).

Anal. Calcd. for $C_{12}H_{14}O_2N_2Cl_2$: N, 9.69

Calcd. for $C_{12}H_{11}O_2NCl_2$: N, 5.15

Found: N, 12.27, 12.08

The compound did not appear to be ethyl β-formylpropionate 2,4-dichlorophenyl hydrazone or ethyl 3-(5,7-dichloroindole)-acetic acid, two compounds that this reaction might have been expected to yield. The product was not characterized further.

Attempted cyclization of β-formylpropionic acid 2,4- dichlorophenylhydrazone

(I) A solution of 1.0 g. (0.038 mole) of the acid hydrazone in 20 ml. of 20% sulfuric acid in absolute ethanol was refluxed for two hours. The solution was diluted with 300 ml. of water and extracted with three 100 ml. portions of ether. The combined ether extracts were dried with "Drierite" and the ether was distilled off leaving a residue which was saponified with methanolic potassium hydroxide. The methanol was distilled off after the addition of 50 ml. of water and the basic solution was extracted once with ether, which was discarded. Acidification of the basic solution and extraction with ether gave a residue of 0.7 g. after the ether was dried and distilled off. Recrystallization from benzene gave a m.p. of 182-184° (dec.). Recrystallizations from benzene and then from ethyl acetate raised the m.p. to 185-185.5° (cor.). A mixed melting point with β-formylpropionic acid 2,4-dichlorophenylhydrazone, m.p. 181-182° (dec.) gave a m.p. of 182-183° (dec.). The acid hydrazone was recrystallized from benzene and again from ethyl acetate which raised the m.p. to 185-185.5° (cor.) (dec.).

(II) A 2.8 g. portion of acid hydrazone prepared from

2.28 g. (0.0155 mole) of glutamic acid and 3.3 g. (0.0155 mole) of 2,4-dichlorophenylhydrazine hydrochloride was recrystallized from a mixture of ether and benzene and gave a first crop of crystals weighing 1.6 g., m.p. 163-166° (dec.). This material was treated with 30 ml. of 20% sulfuric acid in absolute ethanol. The reaction mixture was left at room temperature for two weeks because of illness. At this time a crystalline deposit had settled out. The reaction mixture was refluxed for two hours, and when cooled a crystalline deposit again appeared. The crystals were separated and washed twice with absolute ethanol: 0.4 g. was obtained, m.p. 81.5-82.5°. Recrystallization from ethanol yielded long needlelike crystals which melted sharply at 84.5-85° (cor.).

Anal. Found: N, 7.71; 7.82

The mother liquor was worked up as usual and 0.3 g. of the starting material, β-formylpropionic acid 2,4-dichlorophenylhydrazone was recovered.

Attempted Preparation of 3-(7-Chloroindole)-acetic Acid

β-Formylpropionic acid o-chlorophenylhydrazone

A hot solution of 15 g. (0.0845 mole) of o-chlorophenylhydrazine hydrochloride, 8.2 g. (0.1 mole) of sodium acetate, and 50 ml. of acetic acid in 150 ml. of water was added to

380 ml. of a solution containing the β-formylpropionic acid formed from 12.5 g. (0.0845 mole) of glutamic acid. A yellow crystalline precipitate mixed with a tarry brown solid was filtered off and the mixture was recrystallized from benzene. The first crop of crystals obtained, 2.8 g., melted at 176.5-178.5° (dec.). Recrystallization from benzene gave crystals melting at 180-181.5° (cor.).

Anal. Calcd. for $C_{10}H_{11}O_2N_2Cl$: N, 12.37

Found: N, 12.33, 12.22

Attempted cyclization of β-formylpropionic acid o-chlorophenylhydrazone

A solution of 30 ml. of absolute ethanol, 5 ml. of sulfuric acid and 2.7 g. (0.012 mole) of the acid hydrazone was refluxed for two hours. The solution was diluted with 500 ml. of water and extracted with four 100 ml. portions of ether. The combined extracts were dried with "Drierite" and the ether removed by distillation. A red brown oil was obtained as a residue and was refluxed with 40 ml. of 10% methanolic potassium hydroxide for one half hour. The methanol solution was poured into 500 ml. of water and the solution was extracted with ether which was discarded. Acidification and extraction of the aqueous solution yielded 2.6 g. of solid after the ether solution was dried and the ether removed. The material proved to be the starting material,

m.p. 180-181.5° (dec.). When mixed with the p-chlorophenylhydrazone of β-formylpropionic acid the m.p. was 179-181° (dec.).

Determination of the Plant Growth Activity of Substituted 3-Indoleacetic Acids

Method

The Pea Test of Went (113, 117) was employed to test the substituted 3-indoleacetic acids that had been prepared, for plant growth activity.

Alaska peas (obtained from the Michael-Leonard Co.) were sterilized by washing with 95% ethanol and then with 0.1% mercuric chloride. The peas were washed well and soaked in water for six hours. The peas were planted in moist sand and grown in total darkness at room temperature for eight days. At this time the plants had developed two nodes with scales and a node at the top bearing a leaf. The top was cut off about 5 mm. below the terminal bud and the stem was split lengthwise with a sharp razor blade for a distance of about 25 mm. The stem was cut off 5 mm. below the slit, and the split section was placed in a petri dish containing 20 ml. of the solution to be tested. Five to eight split sections were placed in each petri dish. The plates were stored in the dark for about twelve hours at which time the plants

were shadowgraphed on bromide paper. According to Went, the curvatures reach a maximum after six hours and remain unchanged. If there is no growth hormone present the two longitudinal sections will curve away from each other, while if an active substance is present the free ends will start to bend inward after about an hour. The outward growth is due to tissue tension while the inward curvature, caused by active growth substances, is a differential growth phenomenon of complex nature.

Stock solutions of the compounds were prepared by weighing small samples on a microbalance, adding an equivalent amount of sodium hydroxide, and diluting with enough water to make the concentration 20 mg. of acid per liter. The sodium salts rather than the acids were used in order to keep the pH above 4, as the test would fail if the solutions were too acidic.

Glass distilled water was used for all dilutions and for rinsing the glassware used, since Went reported that very small amounts of metallic ions seemed to inhibit the curvatures.

Results

The sodium salts of 3-indoleacetic, 3-(2-methyl-7-nitroindole)-acetic, 3-(2-methyl-7-chloroindole)-acetic, 3-(2-methyl-5-chloroindole)-acetic, and 3-(2-methyl-5,7-dichloro-

indole)-acetic acids were tested at concentrations equivalent to 20.0, 10.0 5.00, 2.00 and 1.00 mg. of the acid per liter. The results are shown in Table 2, and in Figures 1, 2, 3 and 4.

Table 2

Plant Growth Activity of Substituted 3-Indoleacetic Acids

Substance[1]	Concentration in mg. of acid per liter				
	20	10	5	2	1
3-Indoleacetic acid	+	+	+	+	+
3-(2-Methyl-5-chloroindole)-acetic acid	+	+	+	+	+
3-(2-Methyl-7-chloroindole)-acetic acid	+	+	+	-	-
3-(2-Methyl-5,7-dichloroindole)-acetic acid	+	+	-	-	-
3-(2-Methyl-7-nitroindole)-acetic acid	-	-	-	-	-

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

Since 3-(2-methyl-5-chloroindole)-acetic acid was active in the lowest concentration, another test was made using lower dilutions and comparing its activity with that of 3-indoleacetic acid. The results of this test are shown in Table 3 and in Figures 5 and 6.

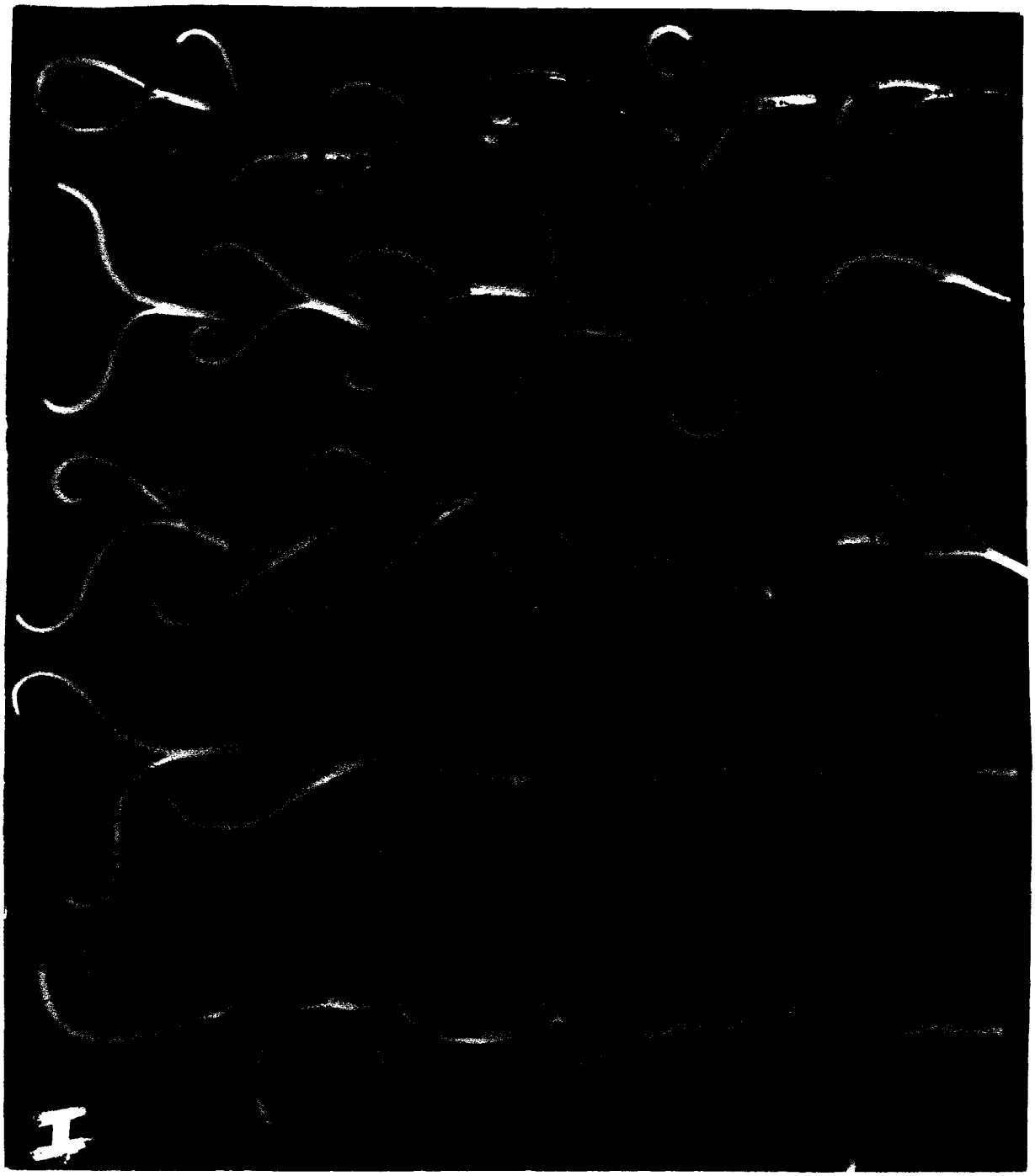


Fig. 1. Curvature of split stems of pea seedlings after 12 hours in sodium 3-indoleacetate solutions. Concentrations, top to bottom rows: 20.0, 10.0, 5.00, 2.00 and 1.00 mg. of 3-indoleacetic acid per liter.

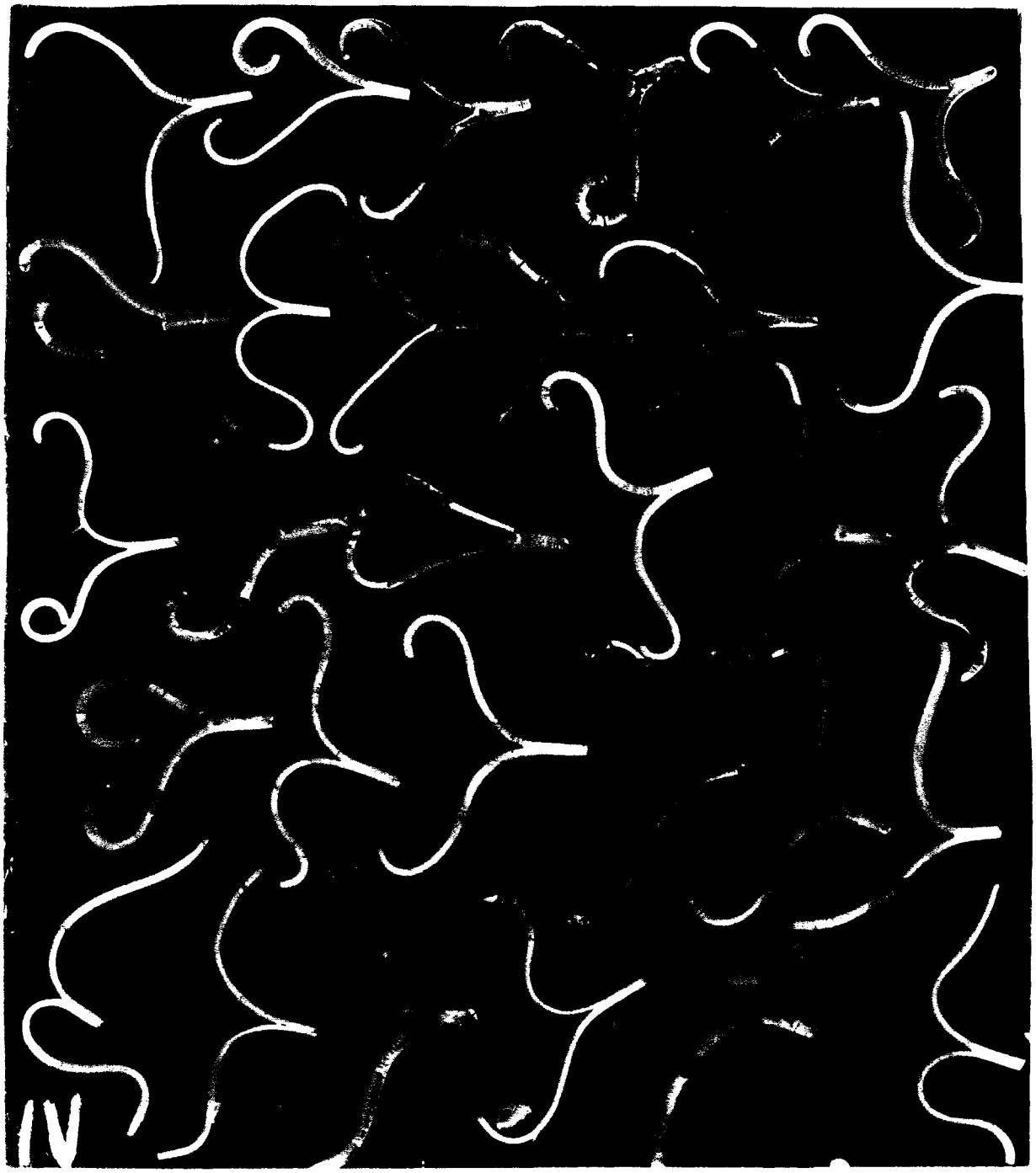


Fig. 2. Curvature of split stems of pea seedlings after 12 hours in sodium 3-(2-methyl-5-chloroindole)-acetate. Concentrations, top to bottom rows: 20.0, 10.0, 5.00, 2.00 and 1.00 mg. of 3-(2-methyl-5-chloro-indole)-acetic acid per liter.

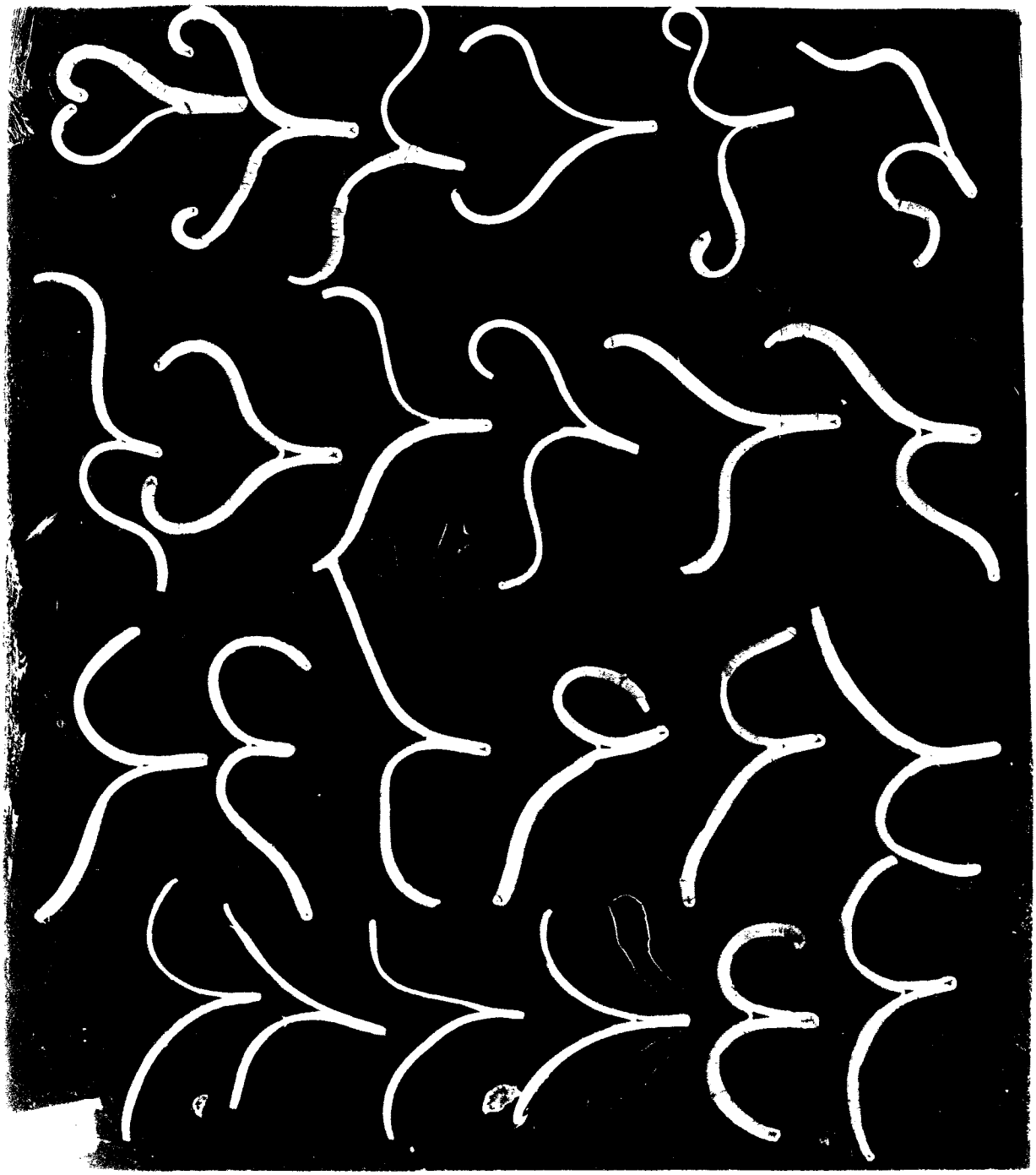


Fig. 3. Curvature of split stems of pea seedlings after 12 hours in sodium 3-(2-methyl-7-chloroindole)-acetate. Concentrations, top to bottom rows: 20.0, 10.0, 5.00, 2.00 and 1.00 mg. of 3-(2-methyl-7-chloroindole)-acetic acid per liter.

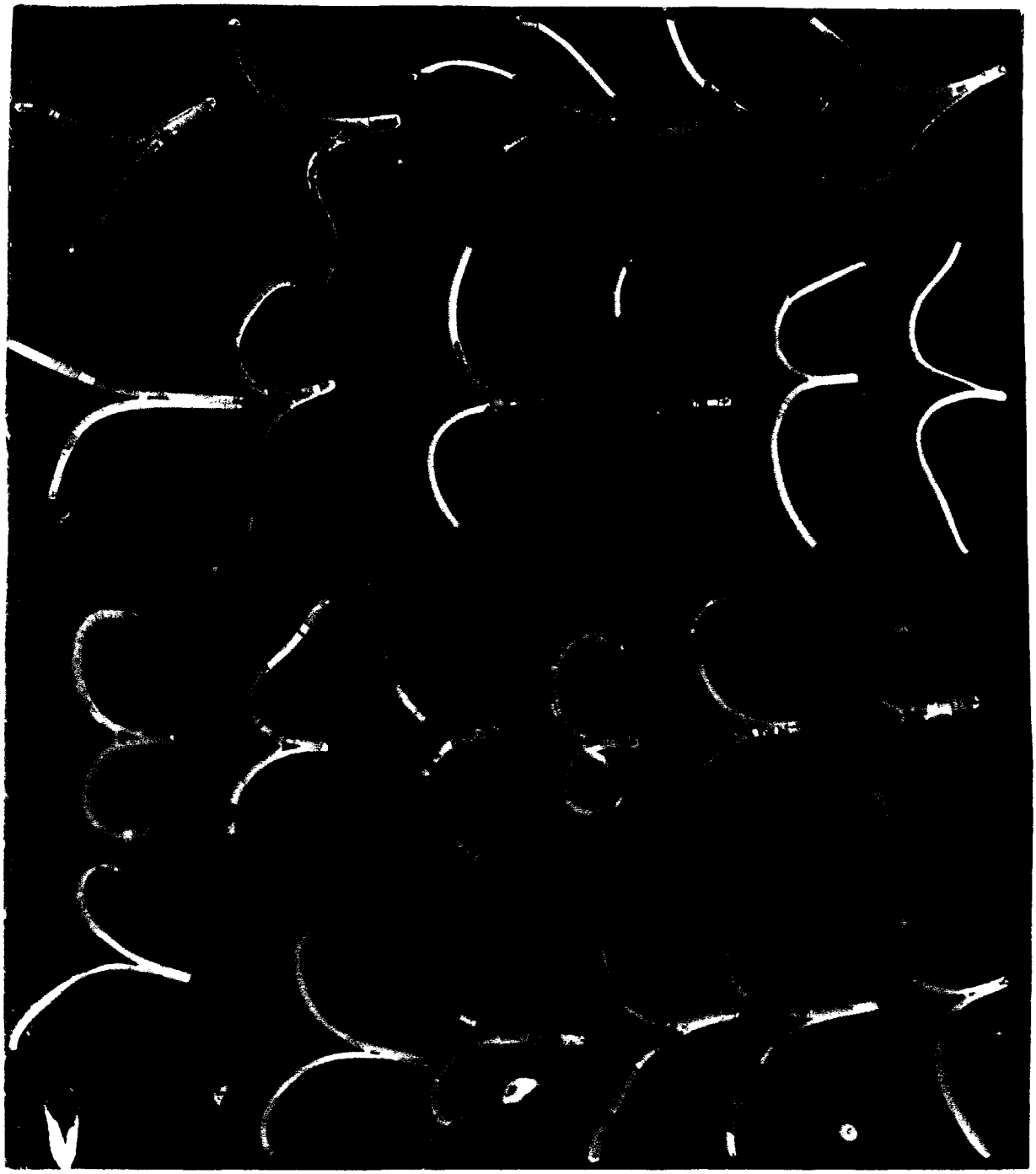


Fig. 4. Curvature of split stems of pea seedlings after 12 hours in sodium 3-(2-methyl-5,7-dichloroindole)-acetate. Concentrations, top to bottom rows: 20.0, 10.0, 5.00 and 2.00 mg. of 3-(2-methyl-5,7-dichloro-indole)-acetic acid per liter.

Table 3

Plant Growth Activity of 3-Indoleacetic and 3-(2-Methyl-5-chloroindole)-acetic Acids

Substance[1]	Concentration in mg. of acid per liter					
	2.00	1.00	0.50	0.25	0.10	0.01
3-Indoleacetic acid	+	+	+	+	+	-
3-(2-Methyl-5-chloro-indole)-acetic acid	+	+	-	-	-	-

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

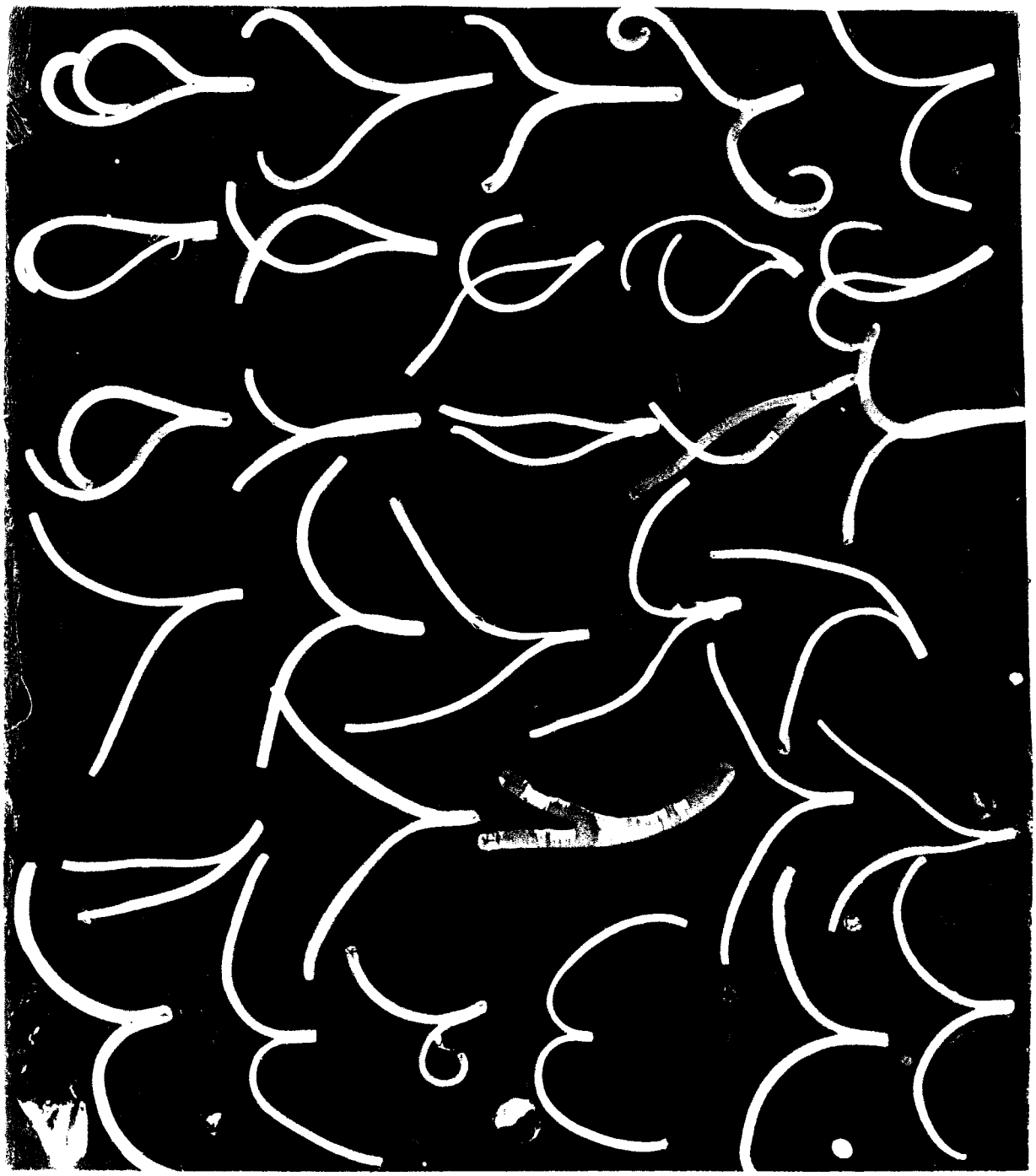


Fig. 5. Curvature of split stems of pea seedlings after 12 hours in sodium 3-indoleacetate. Concentrations, top to bottom rows: 2.00, 1.00, 0.50, 0.25, 0.10 and 0.010 mg. of 3-indoleacetic acid per liter.

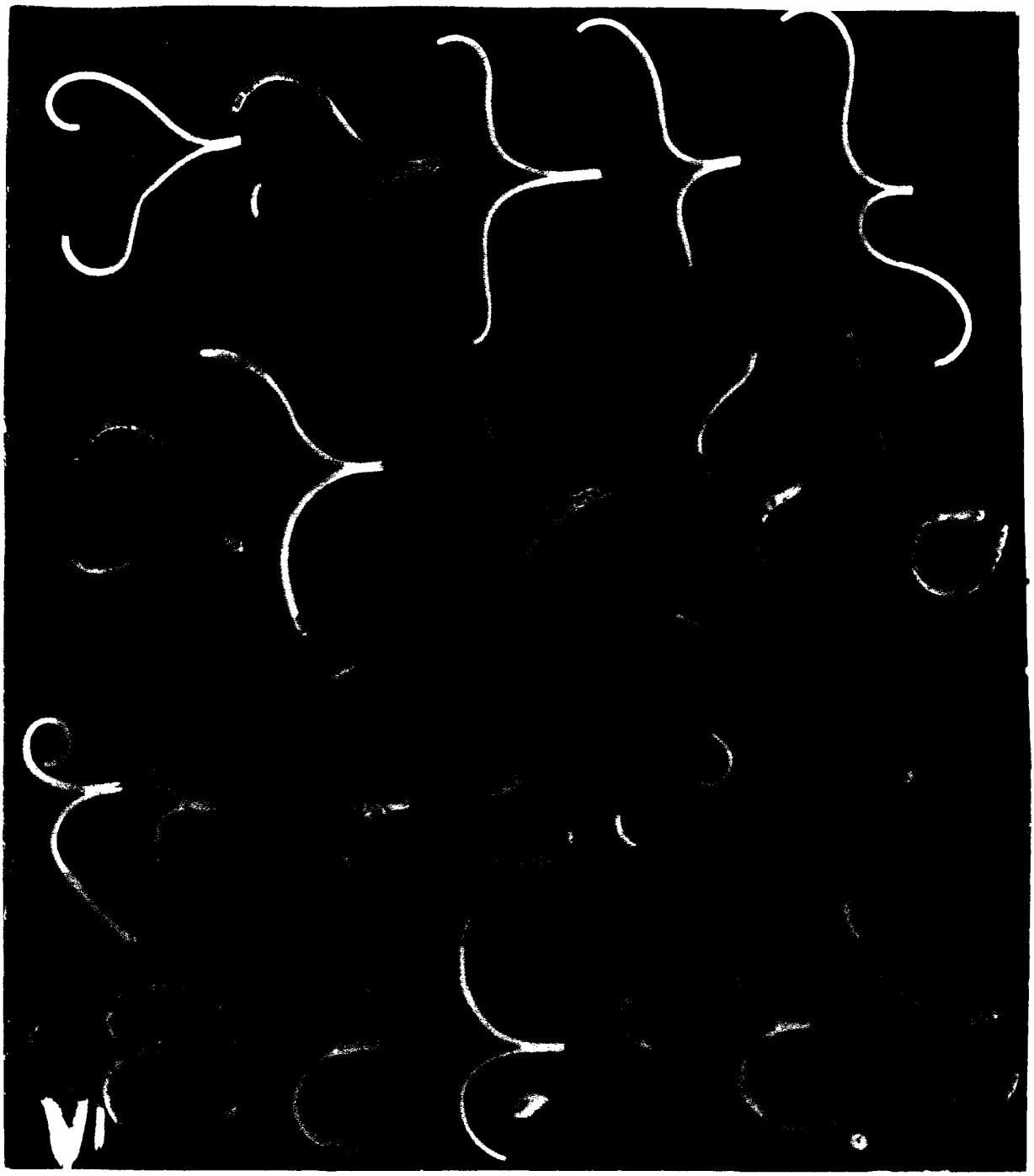


Fig. 6. Curvature of split stems of pea seedlings after 12 hours in sodium 3-(2-methyl-5-chloroindole)-acetate. Concentrations, top to bottom rows: 2.00, 1.00, 0.50 and 0.25 mg. of 3-(2-methyl-5-chloroindole)-acetic acid per liter.

Although the Pea Test for hormone determination was only roughly quantitative the results indicate that substitution in the nucleus does effect the activity of 3-(2-methylindole)-acetic acid. The order of activity found for the chloro derivatives, 3-(2-methyl-5-chloroindole)-acetic acid > 3-(2-methyl-7-chloroindole)-acetic acid > 3-(2-methyl-5,7-dichloroindole)-acetic acid, did not parallel that found by Hitchcock (124) for the phenoxyacetic series, 2,4-dichlorophenoxyacetic acid > p-chlorophenoxyacetic acid > o-chlorophenoxyacetic acid.

DISCUSSION

Chemical

As this discussion will follow the chronological order of the Experimental, the preparation of β-formylpropionic acid will be considered first. The preparation of this compound from glutamic acid by oxidation with sodium hypochlorite seemed to be greatly influenced by the concentration of the reactants and the temperature at which the reaction was carried out. Increasing the temperature to boiling lowered the yield, while increasing both the temperature and the concentration of glutamic acid resulted in a still smaller yield. Moderate yields of 60-65% were obtained from relatively large amounts (0.2 mole) of glutamic acid by carrying out the decomposition of the intermediate N-chloro-α-aminoglutaric acid at 50°. Langheld (78) had reported a higher yield (92%), but his work was carried out on smaller quantities (0.01 mole) in dilute solution.

Dakin's method (16), utilizing Chloramine-T, could also be used for the oxidation reaction at 50°.

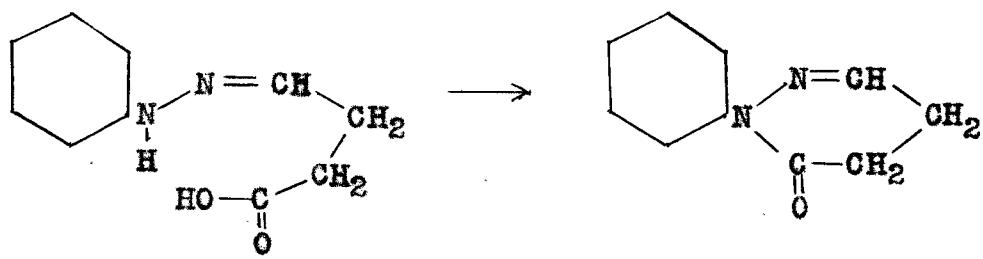
Isolation of the free β-formylpropionic acid was not accomplished. All attempts to prepare it led to resinous materials of high melting point, which were probably higher

polymers of the type reported by Carriére (13), who obtained a trimer of β-formylpropionic acid, and by Ungern-Sternburg (108), who had obtained a dimer. In the present investigation the isolation of the free β-formylpropionic acid was unnecessary since the aqueous solutions of the substance could be used for preparing the substituted phenylhydrazones that were required.

Experiments carried out in an attempt to convert glutamic acid into 3-indoleacetic acid using the reaction discussed above coupled with the Fischer Indole Synthesis met with reasonable success. A thirty per cent yield based on glutamic acid was realized in one experiment which utilized alcoholic sulfuric acid as the cyclizing agent. It is conceivable that the yield could be increased considerably by using improved or new methods for the cyclization and recovery.

In several of the attempts made to prepare 3-indoleacetic acid, Chloramine-T was used to oxidize the glutamic acid to β-formylpropionic acid. The phenylhydrazone prepared from this reaction was contaminated with p-toluenesulfonamide which had not been completely removed previously in a filtration. This p-toluenesulfonamide caused considerable difficulty in the purification procedure. An attempt was made therefore to purify the oily phenylhydrazone by vacuum distillation. When the material was heated under re-

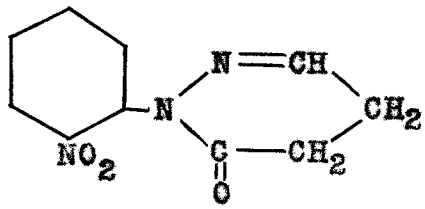
duced pressure, a reaction took place giving a liquid which distilled and solidified. The product appeared to be of the same type as the compound that Fischer (24) obtained when he heated the phenylhydrazone of levulinic acid at an elevated temperature. The reaction involves the splitting out of a molecule of water from the compound to form what Fischer called an anhydride, since alkaline hydrolysis yielded the original acid.



An attempt to prepare 3-(5-nitroindole)-acetic acid from β-formylpropionic acid p-nitrophenylhydrazone using alcoholic sulfuric acid as the cyclizing agent was unsuccessful. Further attempts to prepare this compound were not made in the present investigation. However, failure with one reagent does not necessarily mean that ring closure will not occur. In fact, other investigators (51, 82) have found that certain ring closures are accomplished only by a certain ring closures are accomplished only by a certain reagent which fails completely for the ring closure of other phenylhydrazones.

β-formylpropionic acid o-nitrophenylhydrazone was subjected to cyclization by concentrated sulfuric acid, and a compound was obtained which appeared to be the anhydride of

the o- nitrophenylhydrazone of β-formylpropionic acid.



Treatment of the hydrazone with alcoholic hydrogen chloride was also unsuccessful. The product obtained was the ethyl ester of the hydrazone.

Cyclization of the ethyl β-formylpropionate o-nitrophenylhydrazone with zinc chloride was attempted, but the very small amount of material recovered was not enough to carry the investigation further.

Attempts to prepare some substituted 3-(2-methylindole)-acetic acids were more successful. Alcoholic hydrogen chloride yielded the ethyl ester when reacted with levulinic acid o-nitrophenylhydrazone; the same compound was obtained from ethyl levulinate and o-nitrophenylhydrazine. Cyclization of ethyl levulinate o-nitrophenylhydrazone with zinc chloride, however, resulted in the formation of the desired product, 3-(2-methyl-7-nitroindole)-acetic acid. Further investigation showed that the acid could be prepared in better yield by using hydrochloric acid saturated with zinc chloride for closing the ring. This reagent had not been used previously.

The preparation of three derivatives of 3-(2-methyl-

indole)-acetic acid substituted with chloro- groups in the 5-, 7-, and 5,7- positions was accomplished relatively easily in 30-40% yields. The preparation of the analogous 7- and 5,7-dichloro- derivatives of 3-indoleacetic acid was not successful with the cyclizing agents that were tried.

Physiological

The substituted 3-indoleacetic acids that were prepared were tested for plant growth activity by the Pea Test of Went (113, 117). In these tests the 3-(2-methyl-7-nitro-indole)-acetic acid was completely inactive. This is in accordance with the observation of Zimmerman and Hitchcock (125) that p-nitrophenoxyacetic acid is completely inactive. The effect of a substituted group seems to depend not only on the position it occupies but also upon the chemical nature of the group itself. p-Chlorophenoxyacetic was eighty times as active as phenoxyacetic acid as a plant hormone.

The substituted chloro- derivatives of 3-indoleacetic acid were all active to a variable degree as shown in Table 4.

Table 4

Comparison of the Plant Growth Activity of Some 3-Indoleacetic Acids

Substance	Lowest concentration of acid showing growth activity
3-Indoleacetic acid	0.1 mg. per liter
3-(2-methyl-5-chloroindole)-acetic acid	1.0 mg. per liter
3-(2-methyl-7-chloroindole)-acetic acid	5.0 mg. per liter
3-(2-methyl-5,7-dichloroindole)-acetic acid	10.0 mg. per liter

Since 3-(2-methylindole)-acetic acid was not tested no definite conclusions can be drawn at the present time about the effect of substituted chloro- groups. Methyl 3-(2-methylindole)-acetate was found to be inactive in the Pea Test by Haagen-Smit and Went (42), and Kögl and Kostermans (77) had shown that 3-(2-methylindole)-acetic acid was 0.5% as active as indoleacetic acid in the Avena test. The difference in activities of the three chloro- derivatives prepared, however, seems to warrant a further study of this type of substitution, particularly with respect to obtaining derivatives of 3-indoleacetic acid itself.

SUMMARY

1. Several substituted 3-indoleacetic acids were prepared by the Fischer Indole Synthesis and tested for plant growth activity. 3-(2-methyl-7-nitroindole)-acetic acid was inactive, and 3-(2-methyl-5-chloroindole)-acetic, 3-(2-methyl-7-chloroindole)-acetic, and 3-(2-methyl-5,7-dichloroindole)-acetic acids were active in the Pea Test for plant hormones.

2. Attempts to prepare 3-(7-nitroindole)-acetic, 3-(5-nitroindole)-acetic, 3-(5,7-dichloroindole)-acetic, and 3-(7-chloroindole)-acetic acids were unsuccessful.

3. Langheld's method was adapted for the preparation of larger quantities of β-formylpropionic acid from glutamic acid. The β-formylpropionic acid can be converted into 3-indoleacetic acid.

4. Some physical constants for the prepared compounds and their intermediates are reported.

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