ABSTRACT

THE RELATIONSHIP OF CHEMICAL STRUCTURE AND PLANT GROWTH REGULATOR ACTIVITY IN INDOL-3-YLACETAMIDES

by Thomas Charles Hageman

The plant growth regulating activity of indol-3-ylacetic acid is well established; numerous derivatives of indol-3-ylacetic acid also exhibit similar physiological activity. Indol-3-ylacetamide, a naturally occurring derivative of indol-3-ylacetic acid, exhibits definite auxin activity in many plant species. Recently, several other simple amides have shown plant growth regulating activity; among these are: N,N-dimethyl-2,2-diphenylacetamide, dimethylpropynylbenzamides, and the dimethylhydrazides of succinic and maleic acids.

The purpose of this study was to determine the effects of N-substitution on the biological activity of indol-3-ylacetamide and to explore the mechanism of action of these compounds. The following compounds were synthesized and bioassayed:

N-methylindol-3-ylacetamide

N,N-dimethylindol-3-ylacetamide

N-ethylindol-3-ylacetamide

N,N-diethylindol-3-ylacetamide

N-(2-chloroethyl)indol-3-ylacetamide

N-propylindol-3-ylacetamide

N,N-dipropylindol-3-ylacetamide

N-(3-chloropropyl)indol-3-ylacetamide

N-isopropylindol-3-ylacetamide

N,N-diisopropylindol-3-ylacetamide

N-cyclohexylindol-3-ylacetamide

N-dimethylaminoindol-3-ylacetamide

indol-3-ylacetanilide

N,N-diphenylindol-3-ylacetamide

N-methylindol-3-ylacetanilide

N-(2-chlorophenyl)indol-3-ylacetamide

N-(3-chlorophenyl)indol-3-ylacetamide

N-(4-chlorophenyl)indol-3-ylacetamide

N-(2,4-dichlorophenyl)indol-3-ylacetamide

N-(2,5-dichlorophenyl)indol-3-ylacetamide

N-(1-naphthyl)indol-3-ylacetamide

N-benzylindol-3-ylacetamide

N,N-dibenzylindol-3-ylacetamide

N-benzyl-N-methylindol-3-ylacetamide

N-(2-chlorobenzyl)indol-3-ylacetamide

N-(3-chlorobenzyl)indol-3-ylacetamide

N-(4-chlorobenzyl)indol-3-ylacetamide

N-(2,4-dichlorobenzyl)indol-3-ylacetamide

N-(3,4-dichlorobenzyl)indol-3-ylacetamide

Thomas Charles Hageman

The following bioassays were used: the Avena straight growth assay, the cucumber root inhibition assay, and the cucumber epicotyl curvature assay. These compounds exhibited a wide range of activity in the bioassays; in general, the phenyl derivatives were the most active, followed by the alkyl derivatives. The benzyl derivatives showed little or no activity. The addition of chlorine usually increased the activity of the compound. A correlation was found between the pK_a's of the free primary amines and the activity of the corresponding amides.

A metabolic study showed that these compounds are hydrolyzed to indol-3-ylacetic acid *in vivo*; there was a positive correlation between the amount of hydrolysis and the biological activity in the Avena assay. These compounds probably derive their activity from their conversion to indol-3-ylacetic acid; N-dimethylaminoindol-3-ylacetamide may be an exception to this theory. N-(3-chlorophenyl)-indol-3-ylacetamide is unusual in that it has high activity at extremely low concentrations in both the Avena assay and the root inhibition assay, and yet it has very low activity in the cucumber curvature assay. This might be a means of localizing the effects of applied auxin solutions on plants.

THE RELATIONSHIP OF CHEMICAL STRUCTURE AND PLANT GROWTH REGULATOR ACTIVITY IN INDOL-3-YLACETAMIDES

by

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TABLE OF CONTENTS

TABLE OF CONTENTS (cont.) Page

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

INTRODUCTION

A fundamental property of living organisms is their ability to control the processes of growth, development, and reproduction. In recent years biologists and biochemists have taken an increasing interest in the control mechanisms of biological systems.

In the plant kingdom growth and development are under the control of a highly complicated hormonal system. There are many types of compounds which are involved in the control processes; their activities, however, appear to be intricately interrelated. The first class to be isolated and characterized were the auxins or indole compounds. Perhaps the most important auxin compound is indol-3-ylacetic acid (IAA). The role of IAA in controlling such widespread phenomenon as flowering, apical dominance, cell elongation, and fruit setting is well established.

Numerous derivatives of IAA have been synthesized and studied in regard to plant growth regulating activity.

A naturally occurring derivative of IAA is indol-3-ylacetamide (IAAm). IAAm exhibits definite auxin activity in a number of plant species (1).

In recent years the tremendous biological activity of some substituted amides has been discovered. N,N-dimethyl-2,2-diphenylacetamide (diphanamid) is a powerful pre-emergent herbicide, it exhibits its activity through its effects on root tissue (2). Another class of pre-emergent herbicides are the dimethylpropynylbenzamides, in particular, 3,5-dichloro-N-(1,1-dimethyl-2-propynyl)-benzamide (Kerb) (3). More recently, the inhibitory effects of the dimethyl hydrazides of succinic acid (B-995) and of maleic acid (C-011) have been reported (4-6). These compounds, which are simple substituted amides, apparently interfere with the regulatory action of auxins and gibberellins (7).

This study was undertaken to establish a better understanding of the growth regulatory activity of substituted amides and in particular indol-3-ylacetamides. The study consists of three parts: the chemical sysnthesis of a series of N-substituted indol-3-ylacetamides, the

characterization of their biological activity, and a brief study of the metabolism of a few representative compounds.

In this way, we hope to establish the effects of structural modification on biological activity, to gain some understanding of the mechanism of action of indol-3-ylacetamides, and to learn more about the metabolism of these compounds.

HISTORICAL

HISTORICAL

Although indol-3-ylacetic acid was first prepared by Ellinger in 1904 (8), it was not until thirty years later that its effects on cell elongation of Avena was shown by Kögl, Haagen-Smit, and Erxleben (9). Proof of its existence as a natural plant product did not come until 1946 when Haagen-Smit isolated and characterized IAA from Zea mays (10).

The amide of IAA was first synthesized in 1925 by treating indol-3-ylacetonitrile with zinc metal in acetic acid (11). In 1940 Baker synthesized the amide through the pyrolysis of the ammonia salt of IAA (12). Subsequent syntheses were devised by Snyder in 1948 (13) and Shaw in 1958 (14). The latter synthesis was the reaction of ammonia with indol-3-ylacetyl chloride, the acid chloride of IAA was first reported by Shaw and Wooley in 1953 (15).

Very little work is reported on the synthesis of N-substituted indol-3-ylacetamides. In the 1950's most of the natural alpha amino acids were used to form amide links

with IAA (16, 17). Aside from these compounds only a few N-substituted amides of IAA have been made; among these are: N,N-dimethylindol-3-ylacetamide (18), N,N-diethylindol-3-ylacetamide (19), N-cyclohexylindol-3-ylacetamide (20), N-benzylindol-3-ylacetamide (21), and indol-3-ylacetanilide (21).

The biological activity of IAAm was first reported by Bentley and Housely in 1952, they proposed that IAAm was an intermediate in the interconversion of IAA and indol-3-ylacetonitrile (22). By the mid-1950's, IAAm was well established as a naturally occurring compound in plant tissue, IAAm was detected by chromatography of extracts of tissue which had been incubated in IAA solutions (23). By the chromatography of extracts of tissue which had been incubated in IAAm solutions, Wain and Wightman showed that the amide is capable of being hydrolyzed to IAA in wheat, pea, tomato, and bean plants (1, 24-25). In 1960, the activities of indol-3-ylcarbonamide, indol-3-ylpropionamide, indol-3-ylbutyramide, indol-3-ylvaleramide, and indol-3-ylcaproamide were compared with those of the parent acids (26). Studies were made on the metabolism of these

compounds by Fawcett, Wain, and Wightman. By using similar incubation and chromatographic techniques as those employed in their previous studies, they found IAA in the extracts from compounds with an even number of carbons in the side chain, and they found indol-3-ylpropionic acid in those from compounds with an odd number of carbons in the side chain. They concluded that the activities of the amides were due to hydrolysis to the parent acid, followed by beta-oxidation to either IAA or indol-3-ylpropionic acid.

None of the N-substituted compounds previously listed were tested for activity as plant growth regulators.

BIOLOGICAL ASSAY

BIOLOGICAL ASSAY

Investigators working with plant growth regulators have devised many biological assays to test the physiological activity of chemical compounds. These assays, using whole plants or excised plant parts, were designed to maximize the desired response and to minimize interfering effects. In general these methods are quite sensitive and reproducible, thus making them a valuable tool in plant hormone studies.

It is important to keep in mind, however, that these assays involve whole plant cells which makes it impossible to distinguish between the primary effects of the compounds acting on the site of action and secondary effects such as metabolism of the compound by the plant as well as factors of transport and penetrability. Bioassays are useful for what they do tell us, that is, the effects of applications of chemicals on a plant, regardless of the mechanism of action.

Three bioassays were used in this experiment; they were: the Avena straight growth, the cucumber root inhibition, and the cucumber epicotyl curvature assay.

The Avena assay is a classic method of studying auxins, the effects of IAA on cell elongation was first established using Avena coleoptiles. In the straight growth assay the ability of the compound to cause cell elongation is measured, the limiting effects of transport and penetration are minimal. Oat coleoptile sections are floated on the solution to be tested, the elongation after twenty-four hours is a measure of activity.

The cucumber root inhibition assay measures the capacity of the compound to inhibit root growth. In all tissue low auxin concentrations stimulate growth and higher than optimal concentrations cause inhibition of growth. Because root tissue has a low optimal auxin concentration they are easily inhibited, thus providing a sensitive test for auxin activity.

The cucumber curvature assay measures the secondary factors of transport and absorption as well as the ability to induce cell elongation in intact plants. Solutions are

applied to one cotyledon of a young cucumber plant, for activity to be observed the compound must be absorbed and transported to the main stem, where active compounds induce a curvature away from the side of application.

Details of the bioassays will be given in the experimental section.

METABOLIC STUDY

METABOLIC STUDY

As was mentioned before, it is impossible to determine from the bioassays alone whether the observed activity is due to the applied compound or if it is an artifact, due to the metabolism of the compound to another more active substance. In particular, Wain and Wightman claim that the activity of IAAm is derived from its hydrolysis to IAA. In their experiments they incubated plant tissues in solutions containing 1000 micrograms of IAAm per 50 ml. of water. When an extract from this solution was chromatographed they obtained a distinct spot corresponding to IAA; a bioassay of a similar chromatograph confirmed the presence of IAA. While Wain and Wightman did show that IAA and IAAm are capable of being metabolically interconverted, their evidence does not conclusively prove that the amount of IAA formed from IAAm is sufficient to evoke the response elicited by the IAAm.

In our study we have compounds having activity greater than IAAm and others having almost no activity

at all. If the activity of the amides is due to their hydrolysis to IAA, then the amount of IAA formed should vary with the activity of the amide tested. Our study, a modification of the procedure used by Wain and Wightman, is an attempt to correlate the amount of IAA in plant tissue with the activity of various amides. This study includes chromatographs which were examined by both chemical developing reagents and biological assays.

Six compounds were chosen for this study; they were picked to give a wide range of activity and type of substitution. The compounds studied are: IAAm, N-methyl IAAm, N,N-dimethyl IAAm, N-methylamino IAAm, N-(2-chloroethyl) IAAm, and N-(3-chlorophenyl) IAAm.

EXPERIMENTAL

EXPERIMENTAL

Synthesis of Compounds

N-substituted indol-3-ylacetamides were prepared by the reaction of indol-3-ylacetyl chloride with the appropriate primary or secondary amines. The indolylacetyl chloride was prepared by treating IAA with phosphorous pentachloride according to the method of Shaw and Wooley (15). This compound is somewhat unstable; it was always reacted with the amines immediately after it was prepared. The reaction with the amines proceeds rapidly in ether solutions at 0°C. The free amines were reacted in a 2:1 molar ratio with the acid chloride. When the amine hydrochlorides were used, they were reacted in a water-ether mixture using sodium hydroxide to absorb the HCl. Pyridine, which is commonly used to absorb HCl in reactions, could not be used because it reacts with indol-3-ylacetyl chloride in ethereal solutions to form an insoluble orange tar (27).

The greatest difficulty encountered involved the purification and crystallization of the products. The

aromatically substituted amides crystallized from methanol, aqueous ethanol, or ethyl acetate. The lower molecular weight aliphatically substituted amides were much more difficult to crystallize; they tend to form an oil from most solvents rather than to crystallize. This problem was overcome by first crystallizing the compounds from the oils, then using some of these crystals to seed the solutions from which the recrystallizations were made.

Indol-3-ylacetyl chloride

Four and 38 hundredths of a gram (0.025 mole) of IAA was dissolved in 150 ml. of anhydrous ethyl ether and the solution was cooled to -5°C in a salted ice-water bath. Five and 73 hundredths of a gram (0.0275 mole) of phosphorous pentachloride was added with stirring in five or six portions over a period of twenty minutes; the reaction was stirred for an additional fifteen minutes. The ether was decanted from the unreacted phosphorous pentachloride and was concentrated to about forty ml. by vacuum, the solution was poured into 400 ml. of petroleum ether (b.p. 30-60°C) which had been previously cooled to -20°C.

After fifteen minutes the precipitate was collected on a Büchner filter and was washed with 50 ml. of petroleum ether. The product was used immediately without further purification. The product melts at 58-60°C with decomposition. Yields ranged from 2.5 to 4.0 gm. (.0129-.0206 mole, 52-83%). The product first forms as white flakes; they quickly turn pink on standing when exposed to atmospheric moisture at room temperature.

N-methylindol-3-ylacetamide

One hundred and fifty ml. of ether, 10 ml. of water, and 2.00 gm. (.0296 mole) of methylamine hydrochloride were mixed and cooled to 0-5°C in a flask equipped with a magnetic stirrer. One and 85 hundredths of a gram (.0280 mole) of potassium hydroxide (85%) was added. With maximum stirring, 3.00 gram (.0155 mole) of indol-3-ylacetyl chloride was added over a period of twenty minutes; the solution was allowed to warm to room temperature as it was stirred for an additional hour. Fifty ml. of water was added, and the solutions were separated. The water fraction was extracted with 100 ml. of ether. The ether fractions were combined, dried over anhydrous sodium sulfate, and evaporated to an

oil residue. The oil was crystallized by cooling in an ice bath and scratching it with a glass rod. The product was recrystallized from methanol and water giving 1.86 gm. (.0099 mole, 64% yield) of yellow prisms. The final product melts at 104-105°C.

Analysis: calculated-- 14.94% N.; found-- 14.81% N.

The following compounds were synthesized in a manner similar to N-methyl IAAm:

Compound	M.P.	Yield	Analysis (%N.) Calculated	Found
N-ethylindol-3-yl acetamide	67-69°	35%	13.85	13.76
N-(2-chloroethyl) indol-3-ylacetamide	93-94°	19%	11.84	11.93
N-(3-chloropropyl) indol-3-ylacetamide	78°	15%	11.17	11.22

N-propylindol-3-ylacetamide

A solution of 2.20 gm. (.0114 mole) of indol-3-ylacetyl chloride in 75 ml. of ether was cooled to -10° C and mixed with a solution of 1.35 gm. (.0228 mole) of propylamine in 75 ml. of ether, which had also been cooled to -10°C. The reaction was stored in the freezer overnight, then the ether was decanted from the precipitate and

evaporated to an oil. The oil was crystallized by scratching at 0°C. The product was recrystallized from methanol and water giving 0.73 gm. melting at 79-82°C. Sixty-three hundredths of a gram melting at 76-80°C were obtained from the precipitate by adding water and extracting it with ethyl acetate. The total yield was 55% (.0063 mole); the final melting point is 93-94°C.

Analysis: calculated-- 12.92% N.; found-- 12.83% N.

The following compounds were synthesized in a manner similar to N-propyl IAAM:

Compound	M.P.	Yield	Analysis (%N.) Calculated	Found
N,N-dimethyl IAAm	115-116°	92%	Lit., 116-7°	126-8°#
N-N-diethyl IAAm	101°	73%	Lit., 101°	(19)
N,N-dipropyl IAAm	79-80°	71%	10.84	10.79
N-isopropyl IAAM	115°	57%	12.92	12.80
N,N-diisopropyl IAAm	211-213°	83%	10.84	10.75

#Fish, Johnson, and Horning synthesized dimethyl IAAm and reported the melting point as 126-8°C; however, they also reported that several crops of dimethyl IAAm melted repeatedly at 116-117°C. The I.R. spectrum of the two samples with different melting point were identical. Our product was recrystallized to a constant melting point using aqueous ethanol and also ethyl acetate (18).

N,N-dibenzylindol-3-ylacetamide

One and five-tenths gram (.00775 mole) of indol-3-ylacetyl chloride was dissolved in 50 ml. of ether and 3.07 gm. (.0156 mole) of dibenzylamine was dissolved in ten ml. of ether. Both solutions were cooled to -20°C and then they were mixed. The reaction was stirred for three hours at 0°C. Then 50 ml. of water was added and the solution was poured into a round-bottom flask; the ether was removed by vacuum, leaving crystals in the water solution. The product was collected on a Büchner filter. The crystals were dissolved in hot ethanol, and water was added. Three grams (.0846 mole, 100% yield) of colorless prisms formed on cooling. Recrystallization was done from ethyl acetate, the final melting point is 155-156°C.

Analysis: calculated-- 7.90% N.; found-- 7.73% N.

The following compounds were prepared in a manner similar to N,N-dibenzyl IAAm:

Compound	M.P.	Yield	Analysis (% N.) Calculated	Found
N-cyclohexyl IAAm	157°	100%	Lit., 155-6°	(20)
N-benzyl IAAm	154-5°	100%	Lit., 152.5 -153.5°	(21)

Compound	M.P.	Yield	Analysis (% N.) Calculated	Found
N-benzyl-N-methyl IAAm	150-1°	93%	10.06	9.92
N-(2-chloro-benzyl) IAAm	161-2%	33%	9.38	9.43
N-(3-chloro-benzyl) IAAm	121°	38%	9.38	9.30
N-(4-chloro-benzyl) IAAm	176-7°	41%	9.38	9.30
N-(2,4-dichoro-benzyl) IAAm	169-71°	54%	8.41	8.54
N-(3,4-dichloro-benzyl) IAAm	167-8°	55%	8.41	8.46
Indol-3-ylacet-anilide	153-4°	100%	Lit.,149.5 -150.0°	(21)
N-methylindol-3-ylacetanilide	134-5°	53%	10.60	10.57
N-(2-chloro-phenyl) IAAm	117-8°	45%	9.84	9.98
N-(3-chloro-phenyl) IAAm	98°	48%	9.84	9.90
N-(4-chloro-phenyl) IAAm	170-2°	67%	9.84	9.88
N-(2,4-dichloro-phenyl) IAAm	152-3°	54%	8.78	8.91
N-(2,5-dichloro-phenyl) IAAm	216-8°	68%	8.78	8.89
N-(1-naphthyl) IAAm	164	83%	9.33	9.22

N,N-diphenylindol-3-ylacetamide

A solution of 2.15 gm. (.0111 mole) of indol-3-ylacetyl chloride in 50 ml. of ether was mixed with 3.75 gm. (.0222 mole) of diphenylamine at room temperature. Unlike the other reactions this reaction is very slow at this temperature; the reaction mixture was left in the dark at room temperature for 48 hours. The ether was decanted from the crystals and evaporated by vacuum. Both fractions were dissolved in ethanol and combined; water was added and 2.24 gm. (.0068 mole, 69% yield) of crystals were obtained. Recrystallization was done with ethyl acetate, the final melting point is 173-175°C.

Analysis: calculated-- 8.58% N.; found-- 8.45% N.

N-dimethylaminoindol-3-ylacetamide

One and 59 hundredths of a gram (.0082 mole) of indol-3-ylacetyl chloride was slowly added to a solution of 0.99 gm. (.0165 mole) of 1,1-dimethyl-hydrazine in 150 ml. of ether and 10 ml. of water. The addition was made at 5°C over a period of twenty minutes; the reaction was stirred for an hour afterward. The ether was separated from the water and was dried over anhydrous sodium sulfate.

The ether was evaporated by vacuum leaving an oil. On the addition of methanol a white powder precipitates (0.20 gm., m.p.141-44°C); the solution was filtered and the methanol was removed by vacuum. The oil was dissolved in ethyl acetate and petroleum ether was added to a slight cloudiness. After two days at -5°C, five-tenths of a gm. (.0023 mole, 28% yield) was obtained; the final melting point is 123-4°C.

Analysis: calculated-- 19.34% N.; found-- 17.95% N.

Biological Assays

Avena Straight Growth (28,29)

Solutions: Solutions of 0.01 molar concentration of the compounds were made by dissolving the compounds in 100% ethanol (8.0% final concentration) and diluted to volume with a pH 5 citrate-phosphate buffer. The buffer was prepared from 20 gm. of sucrose, 1.794 gm. of dipotassium phosphate, 1.019 gm. of citric acid monohydrate, and one ml. of Tween 80 diluted to one liter with glass distilled water. At a 0.01 molar concentration most of the solutions quickly form a milky suspension; the dilutions

were made rapidly before precipitation occurred. The following concentrations were prepared using the citrate-phosphate buffer and were used in the assay: 10^{-3} M, 10^{-4} M, 10^{-5} M, 10^{-6} M, 10^{-7} M, and 10^{-8} M. The buffer was used as the control; 0.8% ethanol in buffer has no effect on the assay results.

Plant Material: Oat seeds (var. Torch) were washed in the dark under running tap water for two hours. They were planted on moist vermiculite and exposed to weak red light for twenty-four hours. They were then covered with one cm. of moist vermiculite and grown in the dark for 48 hours. At this time 5 mm. segments were cut 5 mm. below the tip, a cutting jig was used to insure uniform sections. The coleoptiles were floated for two hours on a solution of one mg. of magnesium sulfate monohydrate per liter of water; only the coleoptiles still floating at this time were used in the assay.

Treatment: One ml. of each solution (10^{-3} M to 10^{-8} M) was put in a test tube with ten coleoptiles. The tubes were put in a near horizontal position on a drum rotating at one revolution per minute; they were incubated

in the dark for 20-24 hours. The coleoptiles were measured by placing them in a photographic enlarger, the shadows were enlarged five times and their lengths were measured to the nearest mm. Each series contained two buffer controls and an IAA standard (10^{-3} M to 10^{-8} M). The data are expressed as percent of control growth and are the mean of three replications.

Cucumber Root Inhibition (30)

Solutions: Solutions of 0.001 molar concentration of the compounds were prepared by dissolving them in a minimal amount of 100% ethanol and diluting them to volume with glass-distilled water. From these solutions the following concentrations were prepared with glass-distilled water: 10^{-4} M, 10^{-5} M, 10^{-6} M, 10^{-7} M, and 10^{-8} M. When the ethanol in the 0.001 molar solution exceeded 0.8%, ethanol solutions were included with the controls, which were glass-distilled water. Eight-tenths of a percent of ethanol had no measurable effects on the results of the assay.

Plant Material and Treatment: Cucumber seeds were germinated in the dark for twenty-four hours on filter

paper moistened with distilled water. Ten seeds with uniform radicals were placed on a filter paper in each Petri dish with 4.5 ml. of the solution to be tested. The dishes were placed in the dark at 25°C, after 48 hours the root length was measured to the nearest mm. Two water blanks and a series of IAA standards were included in each run. The data is the mean of three replications and is expressed as percent inhibition of control.

Cucumber Epicotyl Curvature (29)

Solutions: Solutions of 0.001 molar concentration were prepared by dissolving the compounds in five ml. of 100% ethanol and diluting to 25 ml. with a 1.25% Tween 80 water solution.

Plant Material: Cucumber seeds were grown on moist vermiculite for nine days under fluorescent lights in the laboratory. The plants were then removed from the vermiculite and their roots were washed free from non-plant material with distilled water. They were clamped between two strips of balsa wood (1/8" x 1/8" x 12") held together by rubber bands cut from rubber tubing. The roots were

placed in a 1/3 Hoagland's nutrient solution; they were allowed to equilibrate for at least two hours before treatment.

Treatment: The initial angle of the stem was measured with a protractor equipped with a rotating wire. Ten microliters of the 0.001 molar solutions were placed in the center of one of the cotyledons, after three hours the curvature was again measured in degrees. The control plants were treated with 0.001 M IAA solution; 20% ethanol in one percent Tween 80 does not cause any curvature. The ethanol lessens the response of IAA by less than ten percent. The treatment consists of four replications of five plants each; the data are the mean and are reported as percent of IAA curvature.

Metabolic Study (24)

Oat seeds were soaked and grown under red light as for the Avena assay. They were covered with moist vermiculite and grown for 52 hours at 25°C in the dark. Then, one cm. sections were cut five mm. below the tip, 100 of these

sections were floated on 50 ml. of a 10^{-4} M solution (0.8% ethanol) made with glass-distilled water. Controls included tissue incubated in 50 ml. of glass-distilled water and 50 ml. of the solutions incubated without tissue. The tissue were incubated in the dark for 48 hours at 25°C, then, they were frozen overnight. The next day they were thawed, the tissue was removed, ground to a paste, and recombined with the solution. The pH was adjusted to 12.00 with sodium hydroxide; the solution was extracted with 50 ml. of ethyl acetate. The pH was raised to 2.50 with sulfuric acid and the solutions were extracted three times with 50 ml. portions of ethyl acetate. The ethyl acetate from the acidic extractions was dried over anhydrous sodium sulfate and evaporated to dryness by vacuum. The residue was taken up with a small amount of ethyl acetate and was spotted on a TLC plate (Merk, Silica Gel, F-254, 0.25 mm.). The plate was chromatographed in all glass tanks with n-butanol, ammonia, and water (100:3:18,v/v). After the solvent had moved 15 to 20 cm., the plate was air dried and sprayed with Ehrlich's reagent (1% p-dimethylaminobenzaldehyde in 50% alcoholic HCl).

When the chromatographs were to be examined by bioassay, they were prepared and treated in the same way except they were not sprayed with Ehrlich's reagent. Instead, a section of the silica corresponding to the location of IAA was scraped off of the plate and placed in a test tube. The section included 1.25 cm. either side of a point corresponding to the Rf of the standard of IAA which was chromatographed on the same plate. Five ml. of the Avena buffer was added to the tubes containing the silica. One ml. of this solution and one ml. of a one-in-ten dilution were assayed in the Avena straight growth bioassay. These assays included IAA standards and buffer controls.

RESULTS AND DISCUSSION

RESULTS AND DISCUSSION

Effects of Alkyl Substitution

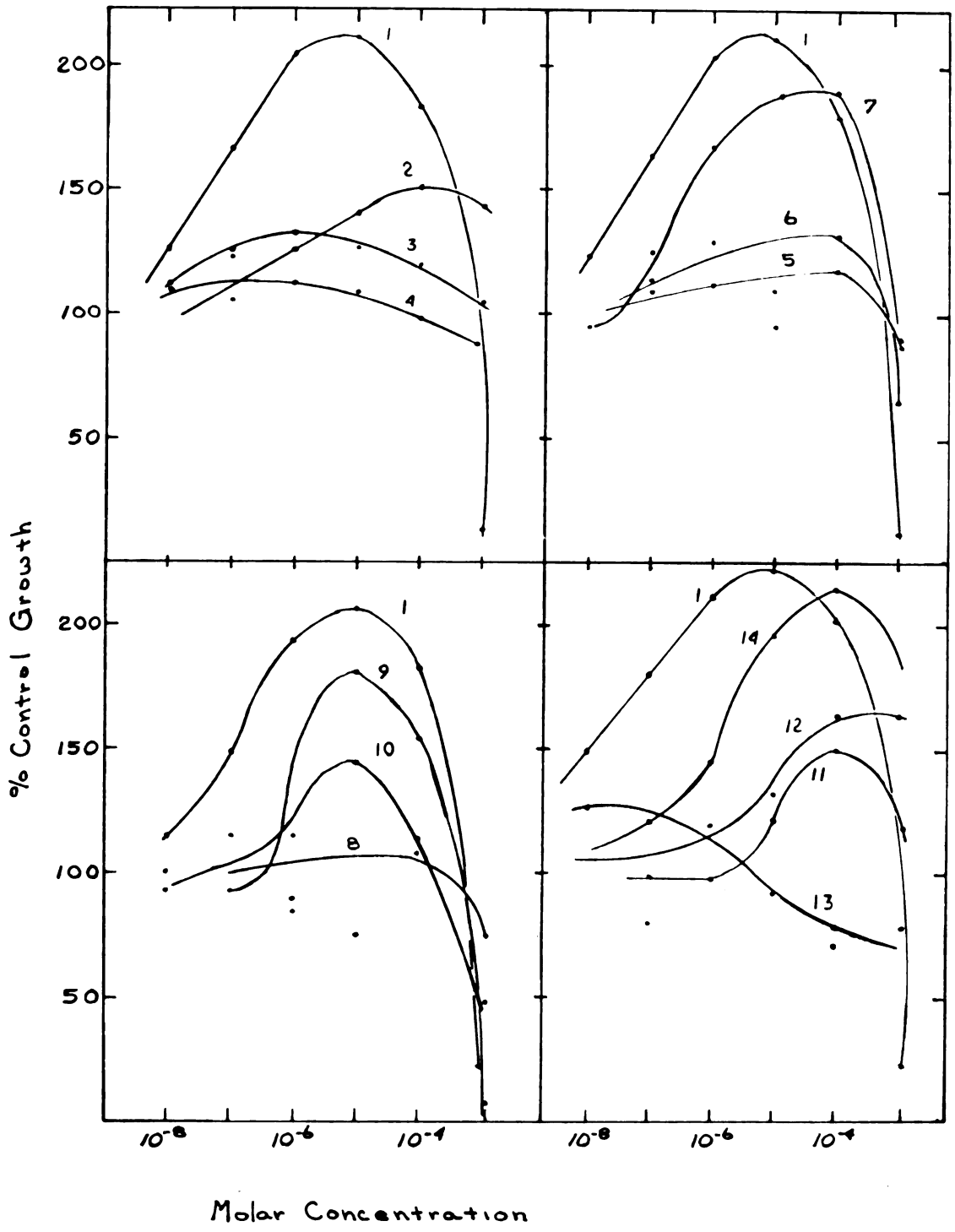
The alkylated derivatives of indol-3-ylacetamide exhibit a wide range of activity. The results of the *Avena* straight growth assay are summarized in Figure I. IAAm itself has moderate activity in this assay; methyl, ethyl, propyl, or cyclohexyl substitution reduces the activity of the amide. The isopropyl derivative has activity about equal to IAAm. The addition of chlorine to the ethyl or propyl derivatives greatly increases the activity of these compounds. Dimethylamino IAAm has activity which is much greater than IAAm.

The di-substituted methyl and ethyl derivatives are inactive, while the di-substituted propyl and isopropyl compounds are more active than IAAm.

In the root inhibition assay, summarized in Table I, IAAm shows significant activity at 10^{-4} M and 10^{-5} M concentrations, the two chlorinated compounds and N-cyclohexyl

Figure I.--Growth Curves of Avena Coleoptile Sections

Effects of Alkyl Substitution-- 1) IAA, 2) IAAm, 3) N-methyl IAAm, 4) N,N-dimethyl IAAm, 5) N-ethyl IAAm, 6) N,N-diethyl IAAm, 7) N-(2-chloroethyl) IAAm, 8) N-propyl IAAm, 9) N,N-dipropyl IAAm, 10) N-(3-chloropropyl) IAAm, 11) N-isopropyl IAAm, 12) N,N-diisopropyl IAAm, 13) N-cyclohexyl IAAm, and 14) N-dimethylamino IAAm.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4
7
6
5
9
10
8
14
12
11
13
10^{-8}
10^{-6}
10^{-4}
Molar Concentration

FIGURE I

TABLE I. ROOT INHIBITION ASSAY--ALKYL DERIVATIVES

Compound	Percent Inhibition					Level of Significance#
	10^{-4} M	10^{-5} M	10^{-6} M	10^{-7} M	10^{-8} M	
IAA	66	19	6	4	-1	11
IAAm	51	20	3	1	3	6
N-methyl IAAm	23	4	2	-4	3	6
N,N-dimethyl IAAm	12	-4	-1	-3	3	6
N-ethyl IAAm	9	-5	0	-2	-3	11
N,N diethyl IAAm	14	5	2	1	-2	11
N-(2-chloro-ethyl) IAAm	73	31	15	8	1	11
N-propyl IAAm	15	-4	-4	2	12	11
N,N-dipropyl IAAm	20	-3	-8	2	-2	11
N-(3-chloro-propyl) IAAm	60	29	4	-5	0	11
N-isopropyl IAAm	19	1	4	2	-3	10
N,N-diisopropyl IAAm	15	3	6	2	4	10
N-cyclohexyl IAAm	32	11	1	1	5	10

#This level is significantly different from zero at the 99.5% confidence level.

IAAm are the only other compounds to cause significant inhibition of root growth at the 10^{-5} M concentration.

The activity of the alkyl derivatives in the cucumber epicotyl curvature assay parallels their activity in the Avena assay (Figure II). There are two notable exceptions, both IAAm and N,N-diisopropyl IAAm exhibit less activity in this assay than would be expected from their activity in the Avena assay. N-cyclohexyl IAAm, which is active in the inhibition of cucumber root elongation, does not exhibit activity in this assay.

Effects of Phenyl Substitution

The addition of an aromatic nucleus next to the amide nitrogen of IAAm greatly increases the auxin activity of these compounds. The results of the Avena assays are given in Figure III. Except for N,N-diphenyl IAAm and N-methylindol-3-ylacetanilide the activity of these compounds is greater than IAAm and in some cases it surpasses the activity of IAA at low concentrations. The diphenyl IAAm is unusual in that its response is

Figure II.--Results of Cucumber Epicotyl Curvature Assay

The curvature induced by each compound is presented as percent of the curvature induced by IAA. Solid bars represent values significantly different from zero with 99% confidence. With the exception of IAA and IAAm, the compounds are designated by the substitution on the amide nitrogen of IAAm.

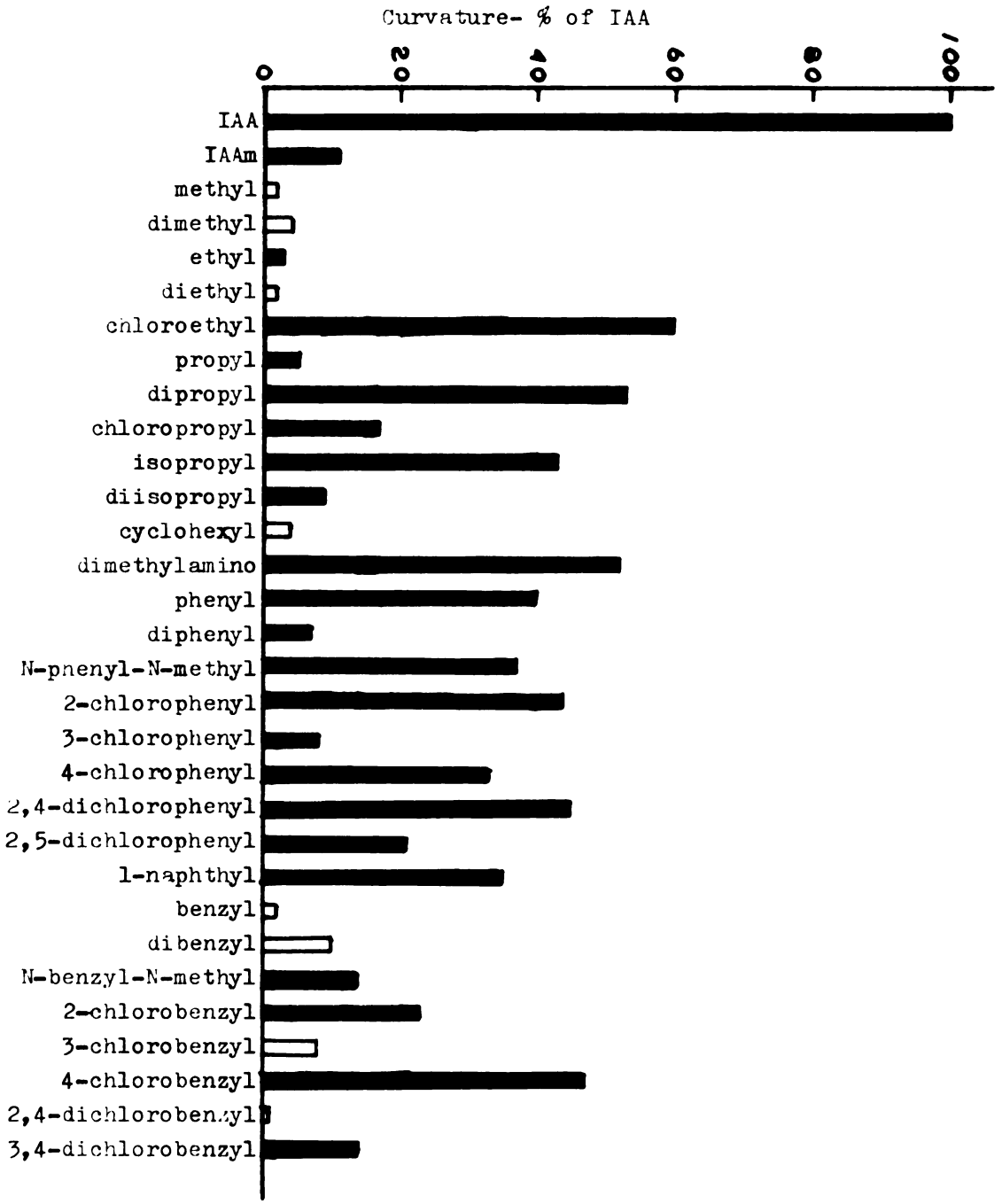

Curvature- % of IAA
0
20
40
60
80
100
IAA
IAAm
methyl
dimethyl
ethyl
diethyl
chloroethyl
propyl
dipropyl
chloropropyl
isopropyl
diisopropyl
cyclohexyl
dimethylamino
phenyl
diphenyl
N-phenyl-N-methyl
2-chlorophenyl
3-chlorophenyl
4-chlorophenyl
2,4-dichlorophenyl
2,5-dichlorophenyl
1-naphthyl
benzyl
dibenzyl
N-benzyl-N-methyl
2-chlorobenzyl
3-chlorobenzyl
4-chlorobenzyl
2,4-dichlorobenzyl
3,4-dichlorobenzyl

FIGURE II.

Figure III.--Growth Curves of Avena Coleoptile Sections

Effects of Phenyl Substitution--1) IAA, 15) indol-3-ylacetanilide, 16) N,N-diphenyl IAAm, 17) N-methylindol-3-ylacetanilide, 18) N-(2-chlorophenyl) IAAm, 19) N-(3-chlorophenyl) IAAm, 20) N-(4-chlorophenyl) IAAm, 21) N-(2,4-dichlorophenyl) IAAm, 22) N-(2,5-dichlorophenyl) IAAm, and N-(1-naphthyl) IAAm.

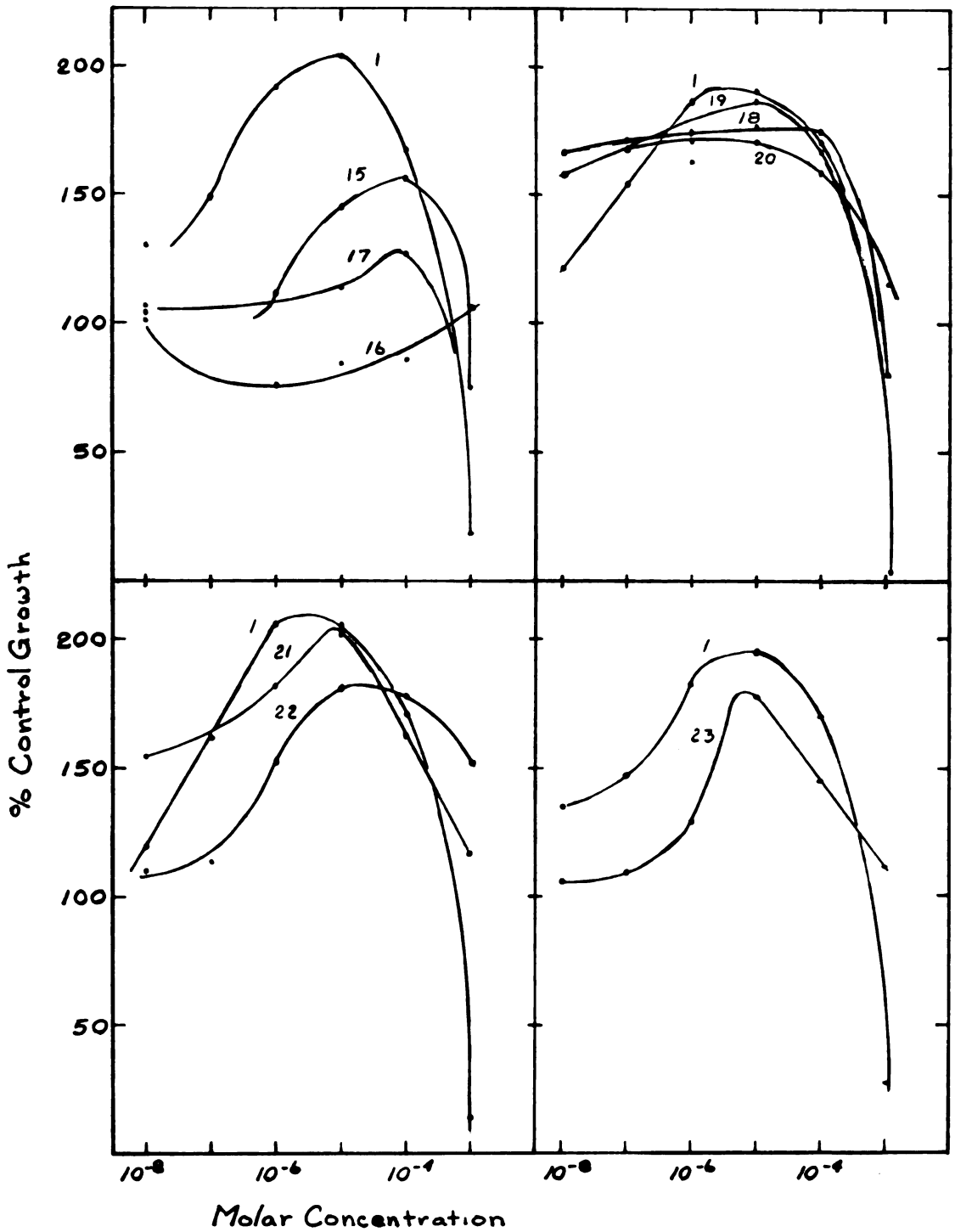

200
150
100
50
% Control Growth
1
15
17
16
19
18
20
21
22
23
10^{-8}
10^{-6}
10^{-4}
Molar Concentration

FIGURE III.

significantly below the control level. Four of the five chlorinated compounds have activity that is greater than 150% of control at the 10^{-8} M concentration. In the mono-chlorinated compounds the position of the chlorine has little effect, but in the dichloro derivatives the position of the chlorine does affect activity. The lack of inhibition at the 10^{-3} M concentration is probably due to the fact that many of these compounds precipitated from solution at this concentration.

The increased activity of the phenyl derivatives also occurs in the root inhibition assay, the data is presented in Table II. Again N,N-diphenyl IAAm and N-methyl-indol-3-ylacetanilide have comparatively low activity, surprisingly N-(2,5-dichlorophenyl) IAAm is inactive in this assay. All of the other compounds cause significant inhibition at the 10^{-6} M concentration; their activity is generally greater than IAA. The position of the chlorine in the mono-chlorinated compounds has little affect in this assay.

TABLE II. ROOT INHIBITION ASSAY--PHENYL DERIVATIVES

Compound	Percent Inhibition 10^{-4} M	10^{-5} M	10^{-6} M	10^{-7} M	10^{-8} M	Level of Significance#
IAA	65	21	12	5	5	9
Indol-3-ylacetanilide	84	69	38	8	3	8
N,N-diphenyl IAAm	3	7	3	4	3	8
N-methylindol-3-yl acetanilide	37	10	7	0	1	8
N-(2-chlorophenyl) IAAm	83	71	32	10	-4	9
N-(3-chlorophenyl) IAAm	82	55	11	4	2	9
N-(4-chlorophenyl) IAAm	81	74	24	4	-8	9
N-(2,4-dichlorophenyl) IAAm	78	64	26	6	-1	9
N-(2,5-dichlorophenyl) IAAm	-8	2	1	-2	-3	9
N-(1-naphthyl) IAAm	62	63	21	7	-3	11

#This level is significantly different from zero at the 99.5% confidence level.

In the curvature assay, N,N-diphenyl IAAm once again has low activity. However, N-methylindol-3-ylacetanilide has much more activity than would be expected. Also unexpected is the low activity of N-(3-chlorophenyl) IAAm, apparently the chlorine in the three position blocks the transport of this compound. The other compounds elicit responses which parallel those of the Avena assay.

Effects of Benzyl Substitution

The placing of a methylene bridge between the aromatic ring and the amide nitrogen of IAAm greatly reduces the activity of these compounds. This reduced activity is evident in the Avena growth curves, Figure IV. Only two compounds have significant activity at concentrations below 10^{-3}M. These are N-benzyl-N-methyl IAAm and N-(4-chorobenzyl) IAAm. In the benzyl derivatives the position of the chlorine does alter the pattern of what little activity that there is.

Figure IV.--Growth Curves of Avena Coleoptile Sections

Effects of Benzyl Substitution-- 1) IAA, 24) N-benzyl IAAm, 25) N,N-dibenzyl IAAm, 26) N-benzyl-N-methyl IAAm, 27) N-(2-chlorobenzyl) IAAm, 28) N-(3-chlorobenzyl) IAAm, 29) N-(4-chlorobenzyl) IAAm, 30) N-(2,4-dichlorobenzyl) IAAm, and 31) N-(3,4-dichlorobenzyl) IAAm.

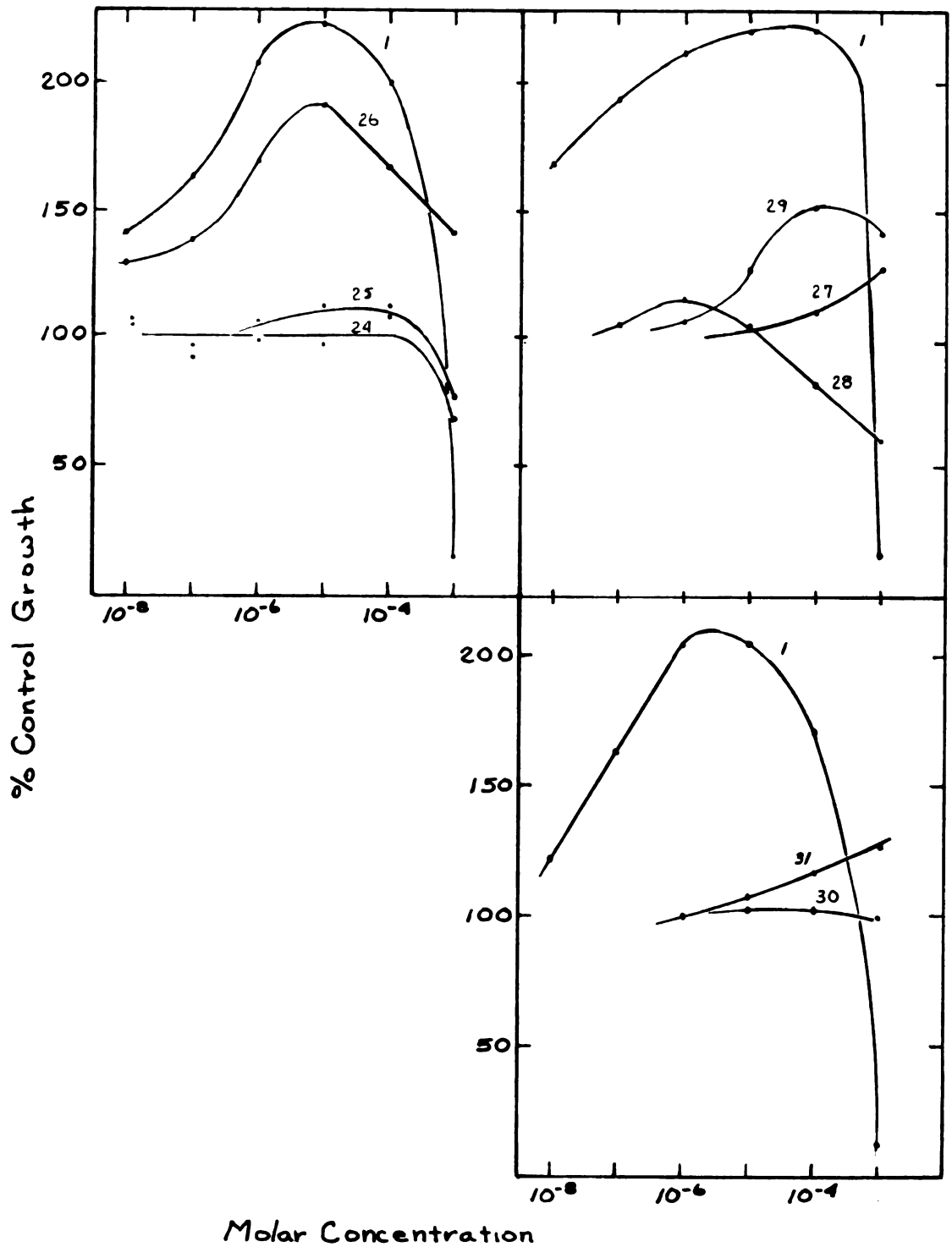

200
150
100
50
% Control Growth
1
26
25
24
29
27
28
31
30
10^{-8}
10^{-6}
10^{-4}
Molar Concentration

FIGURE IV.

The results of the root inhibition assay are given in Table III, the same low level of activity is observed again. N-benzyl IAAm is active as well as the 3-chloro and 4-chloro derivatives. Only N-benzyl IAAm has significant activity at 10^{-5} M concentration. The position of the chlorine is also important in this assay.

In the curvature assay an approximate parallel can again be drawn to the Avena assay.

Metabolic Study

The chromatographs, which were developed by Ehrlich's reagent, showed spots corresponding to IAA in all solutions incubated with tissue, including the water controls. None of the solutions incubated without tissue showed any trace of IAA. The spots were slightly larger and darker in the solutions which contained an amide than those from the water control. However, it was impossible to determine the relative amounts of IAA present from these plates.

TABLE III. ROOT INHIBITION ASSAY--BENZYL DERIVATIVES

Compound	Percent Inhibition					Level of Significance#
	10^{-4} M	10^{-5} M	10^{-6} M	10^{-7} M	10^{-8} M	
IAA	52	16	9	-2	-10	8
N-benzyl IAAm	55	12	0	-6	-1	8
N,N-dibenzyl IAAm	6	1	4	0	1	8
N-benzyl-N-methyl IAAm	0	3	1	-3	-1	8
N-(2-chloro-benzyl) IAAm	7	8	1	1	3	11
N-(3-chloro-benzyl) IAAm	27	1	1	3	4	11
N-(4-chloro-benzyl) IAAm	15	6	1	4	0	11
N-(2,4-dichloro-benzyl) IAAm	9	2	5	-1	-5	11
N-(3,4-dichloro-benzyl) IAAm	0	1	0	-2	-2	11

#This level is significantly different from zero at the 99.5% confidence level.

The results of the biological examination of the plates are given below.

TABLE IV. RESULTS OF METABOLIC STUDY

Treatment of Tissue	Response of *Avena* Assay (% control growth)	
	Original Eluant	1:10 Dilution
Water	65	98
IAAm	79	142
N-methyl IAAm	29	111
N,N-dimethyl IAAm	93	108
N-(2-chloroethyl) IAAm	75	196
N-(3-chlorophenyl) IAAm	90	158
N-dimethylamino IAAm	89	156

The original eluant seems to contain some impurity which obscured the assay results; perhaps this was a trace of the solvent which was absorbed on the silica. The dilution evidently lessened the effects of the impurity so that the relative amounts of IAA could be determined.

Discussion

In these amides there does not appear to be any correlation between steric hinderance about the nitrogen and the activity of the amide. In N,N-dipropyl IAAm and N,N-diisopropyl IAAm the amide nitrogens are very hindered sterically, yet these compounds are more active than IAAm whose nitrogen is completely unhindered. Another compound, N-benzyl-N-methyl IAAm, also has very large groups surrounding the nitrogen; this compound also has more activity than IAAm. This would seem to indicate that the active center of these compounds is not the amide nitrogen but is at least one atom removed.

A significant correlation can be found between the pK_a of the free primary amines and the activity of the corresponding amide in the Avena assay. Figure V is a plot of the response in the Avena assay at 10^{-5} M concentration versus the pK_a of the free amine (31).

The pK_a is the negative logarithm of the equilibrium constant defined by the following equation:

$$K_a = \frac{[B:][H^+]}{[BH^+]}$$

Figure V.--Correlation of pK_a with Avena Straight Growth Activity

A plot of the response of the amides in the Avena assay at the 10^{-5} M concentration versus the pK_a of the corresponding amines. The points are labeled such that the amide is designated by the substitution on the nitrogen (eg. 2,4-Ø-Cl_2 means N-(2,4-dichlorophenyl) IAAm). The line was determined by a least squares analysis, the slope is 8.92; the intercept is 204.

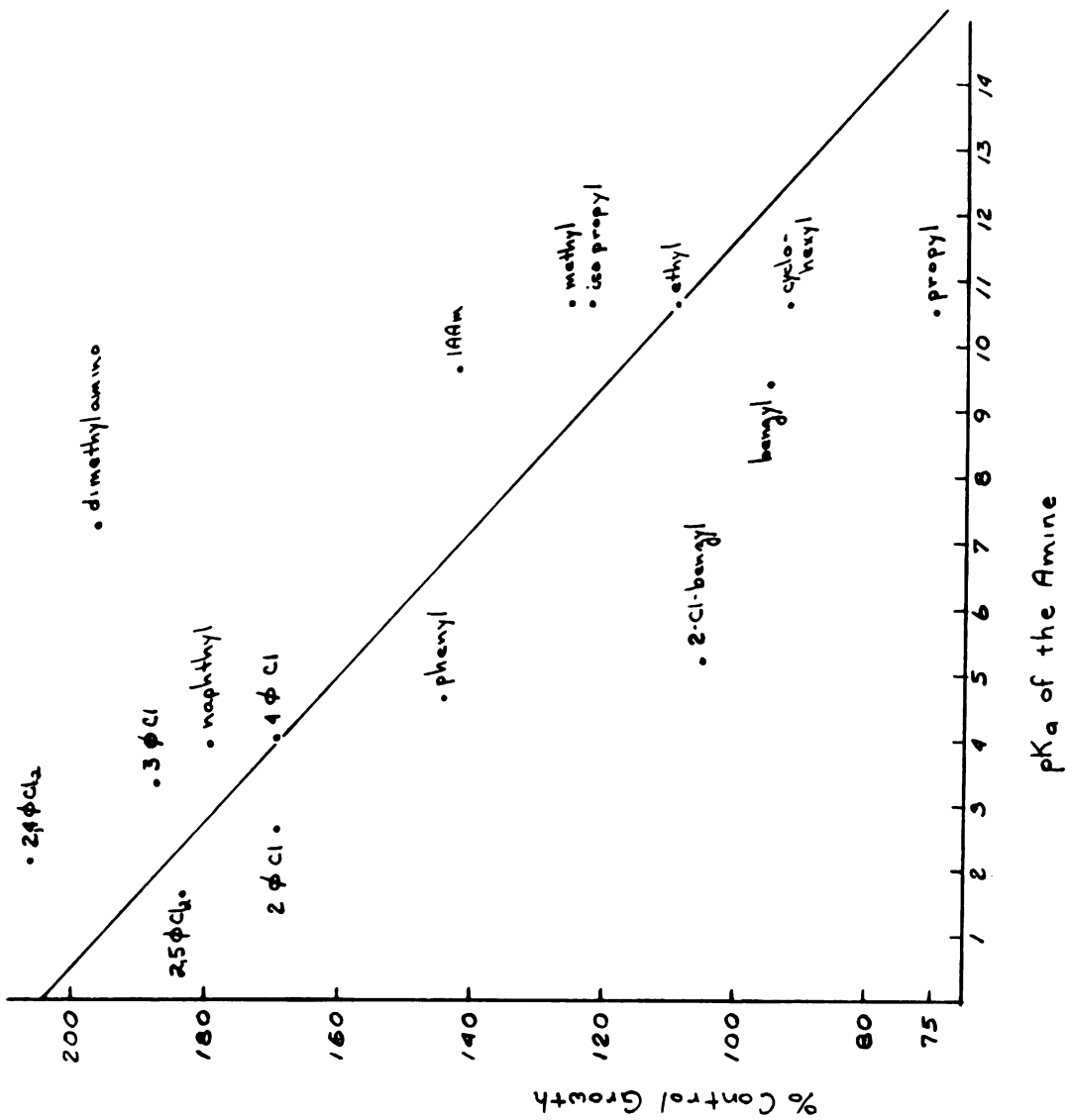

% Control Growth
200
180
160
140
120
100
80
75
1
2
3
4
5
6
7
8
9
10
11
12
13
14
pKa of the Amine
2,4 ΦCl2
3 ΦCl
naphthyl
4 ΦCl
2 ΦCl
2,5 ΦCl2
dimethylamino
IAAm
phenyl
methyl
iso propyl
ethyl
2-Cl-benzyl
benzyl
cyclo-hexyl
propyl

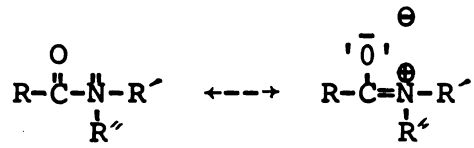
FIGURE V.

The pK_a can be used as a measure of the electron density around the nitrogen in the amine. A high pK_a indicates that the amine is a strong base, that is, the nitrogen is able to easily donate its electrons to bind a proton. Electron donating groups such as alkyl groups strengthen the basicity of the amines. A low pK_a indicates a weak base, the electron density at the nitrogen is lowered by electron withdrawing groups. Phenyl groups are electron withdrawing by induction, chlorine is also highly electronegative.

Figure V clearly shows the relationship between pK_a and activity, the pK_a's of chloroethylamine and chloropropylamine could not be found; however, from inductive effects, their pK_a's would be expected to be higher than the parent amines. This would correspond to the higher activity of these compounds in the bioassays.

Since inductive effects are short range, extending only over two or three atoms, it is obvious that the effects are probably altering the character of the carbonyl of the amide.

Amides can be stabilized by resonance between forms (I) and (II).



(I) (II)

This effect is well known to protein chemists; it plays an important role in the structure of polypeptides. This type of resonance probably occurs in all amides to some degree, depending on the type of N-substitution. Form II would be stabilized by electron-donating groups and destabilized by electron-withdrawing groups. This seems to indicate that the most active amides are those where resonance stabilization is minimal, and the electrons are pulled away from the carbonyl by electron-withdrawing functions.

The results of the metabolic study clearly indicate what the mechanism of action of the amides is and why the effect of pK_a is so pronounced.

When the chromatographic plates were examined using a bioassay it was possible to quantitate the IAA which was extracted and to correlate it to the activity of the compounds. Figure VI shows that there is a very high degree

Figure VI.--Correlation of IAA in Tissue Extract with the Activity in the *Avena* Assay

A plot of the amount of IAA extracted from *Avena* tissue, which had been incubated in amide solutions, as a function of the activity of the amide in the *Avena* assay at 10^{-4} M concentration. The response of an *Avena* bioassay is the measure of the amount of IAA. The points are labeled as in Figure V.

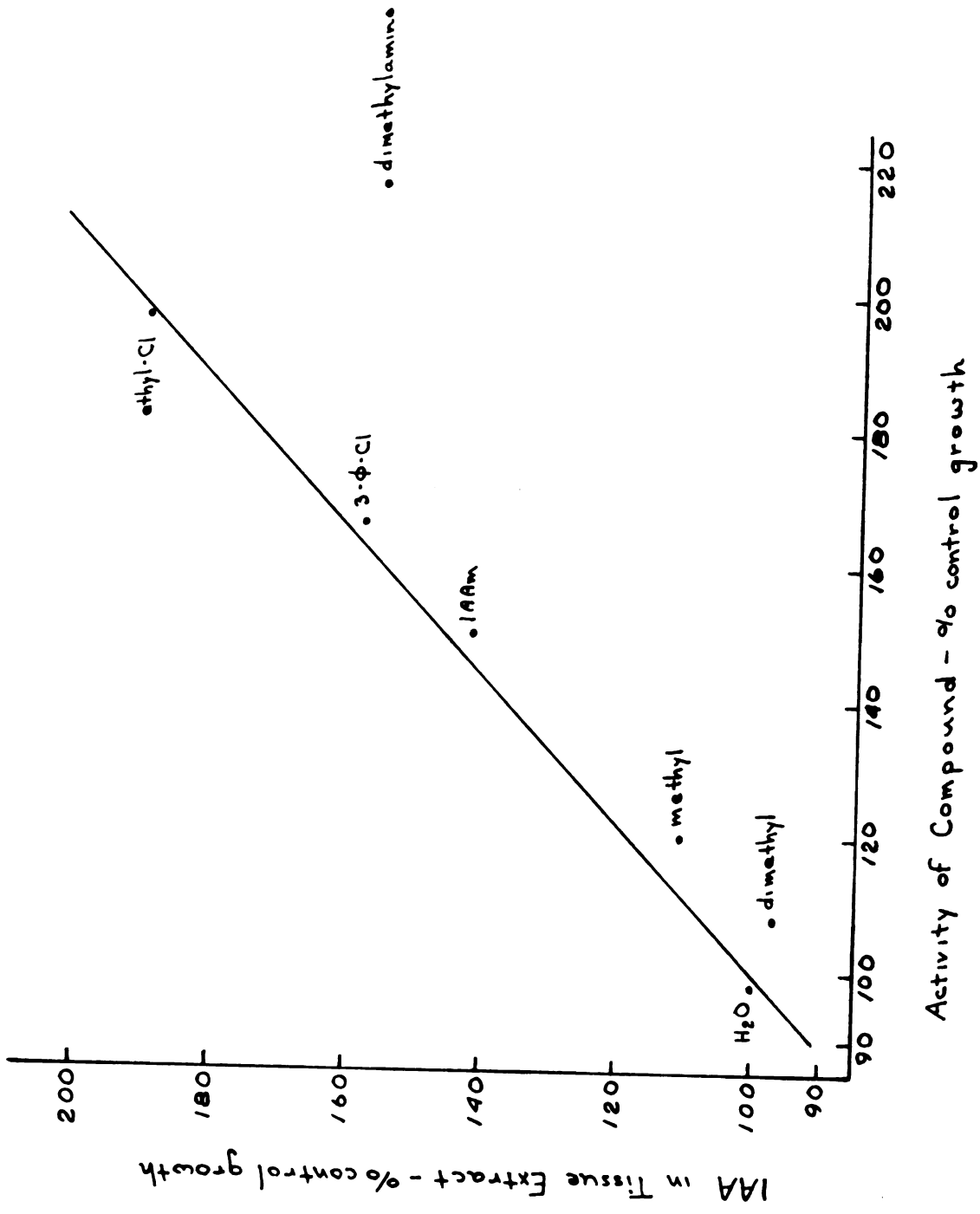

IAA in Tissue Extract - % control growth
200
180
160
140
120
100
90
H2O
dimethyl
methyl
IAAm
3-Φ-Cl
ethyl-Cl
dimethylamine
90 100 120 140 160 180 200 220
Activity of Compound - % control growth

FIGURE VI.

of correlation between these two variables. It is obvious then, that the amides of IAA are not active per se, but their activity is derived from the hydrolysis of the amide to IAA.

The apparent activities would be a function of two rates; first, the rate of entry of the amide into the cell, secondly, the rate of hydrolysis of the amide to the acid. Since these amides are all rather non-polar compounds, their diffusion into the cell would probably be a fairly rapid process. The activity limiting factor would seem to be the rate of hydrolysis. This would agree with the correlation of pK_a to activity; the active center in the hydrolysis step is the carbonyl functionality, not the nitrogen. The development of a partial positive charge on the carbonyl would greatly increase the susceptibility of the amide to hydrolysis.

The high activity of the chlorophenyl IAAm compounds can be readily explained by this model. If the assumption is made that the diffusion of the non-polar amides is more rapid than the diffusion of IAA, which is a polar molecule because of its acid functionality, and

if the hydrolysis of the chlorophenyl IAAm is a rapid process, then the concentration of IAA will be greater in the cells exposed to the amide than the cells exposed to the same level of IAA. This effect would be especially noticeable at low concentrations where the rate of diffusion of IAA approaches the rate of destruction of IAA by metabolic processes.

The activity of the dimethylhydrazide of IAA might not be completely explained by the hydrolysis theory. In both Figure V and Figure VI this compound deviates substantially from the line. In both cases the activity is higher than that predicted by the plot. It is possible that this compound has a different mechanism of action.

The most interesting compound is N-(3-chlorophenyl) IAAm, while it exhibits high activity in both the Avena assay and the root inhibition assay it has very little activity in the cucumber curvature assay. Either it is not transported or it blocks the transport of the IAA produced. Whatever the mechanism, it might have valuable applications in agriculture. Not only is it active at extremely low concentrations, but its effects can be localized to one

section of the plant where it is applied. Further tests would be needed to determine whether this is a general phenomenon and to determine whether the compound is toxic.

SUMMARY

SUMMARY

A series of indol-3-ylacetamides were synthesized and the physiological activity of these compounds in plants was determined. The amides were prepared by the reaction of indol-3-ylacetyl chloride with the appropriate amines. A list of the amides follows:

N-methylindol-3-ylacetamide
N,N-dimethylindol-3-ylacetamide
N-ethylindol-3-ylacetamide
N,N-diethylindol-3-ylacetamide
N-(2-chloroethyl)indol-3-ylacetamide
N-propylindol-3-ylacetamide
N,N-dipropylindol-3-ylacetamide
N-(3-chloropropyl)indol-3-ylacetamide
N-isopropylindol-3-ylacetamide
N,N-diisopropylindol-3-ylacetamide
N-cyclohexylindol-3-ylacetamide
N-dimethylaminoindol-3-ylacetamide
indol-3-ylacetanilide
N,N-diphenylindol-3-ylacetamide
N-methylindol-3-ylacetanilide
N-(2-chlorophenyl)indol-3-ylacetamide
N-(3-chlorophenyl)indol-3-ylacetamide
N-(4-chlorophenyl)indol-3-ylacetamide

N-(2,4-dichlorophenyl)indol-3-ylacetamide

N-(2,5-dichlorophenyl)indol-3-ylacetamide

N-(1-naphthyl)indol-3-ylacetamide

N-benzylindol-3-ylacetamide

N,N-dibenzylindol-3-ylacetamide

N-benzyl-N-methylindol-3-ylacetamide

N-(2-chlorobenzyl)indol-3-ylacetamide

N-(3-chlorobenzyl)indol-3-ylacetamide

N-(4-chlorobenzyl)indol-3-ylacetamide

N-(2,4-dichlorobenzyl)indol-3-ylacetamide

N-(3,4-dichlorobenzyl)indol-3-ylacetamide

The method of synthesis, the yield, and the melting point are given for each compound. A nitrogen analysis was used to characterize those compounds that were not reported in the literature.

Biological assays were used to characterize the physiological activity of the compounds. Three bioassays were used: the Avena straight growth, the cucumber root inhibition, and the cucumber epicotyl curvature assay. The N-alkyl amides displayed a wide range of activity; the most active of these is N-(2-chloroethyl) IAAm. The N-phenyl derivatives generally had very high activity; N-(3-chlorophenyl)IAAm was unusual in that it was highly

active at extremely low concentration in the Avena and root inhibition assays but it was relatively inactive in the cucumber curvature test. These properties might make this compound useful in agriculture. Most of the benzyl derivatives had very low activity in all three assays.

A correlation was found between the pK_a's of the free primary amines and the Avena activity of the corresponding amide. No correlation was readily apparent between activity and steric effects.

A metabolic study showed that these compounds are hydrolyzed to IAA, a correlation was found between the amount of hydrolysis and the activity of the amide. It was postulated that the amides derive their activity from their conversion to IAA; N-dimethylamino IAAm may be an exception to this mechanism. A model was given to explain the high activity of the chlorophenyl derivatives.

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REFERENCES

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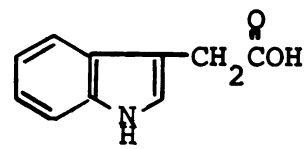
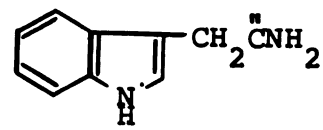
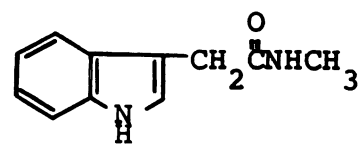
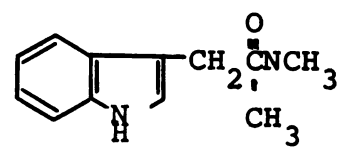
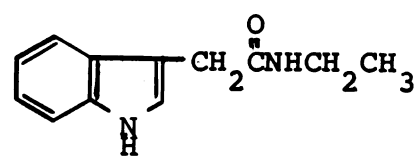
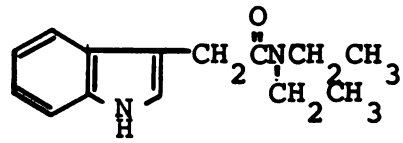
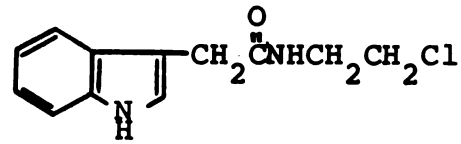
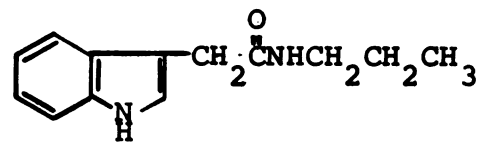
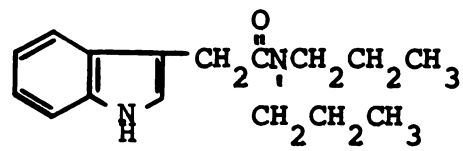
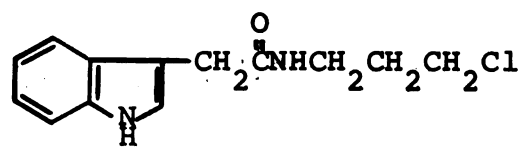
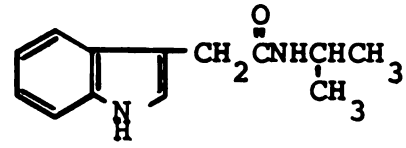
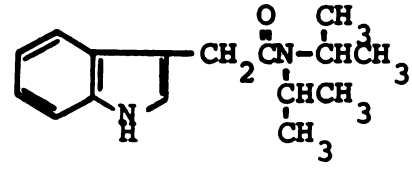
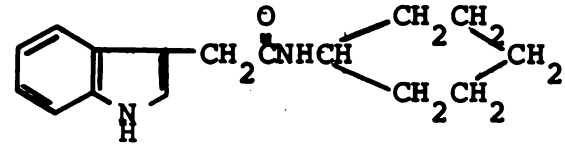
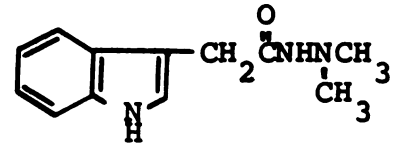
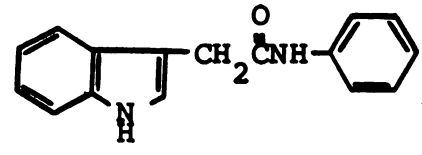
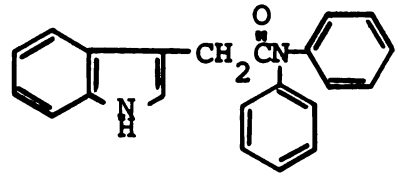
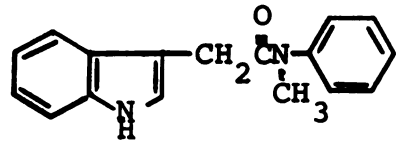
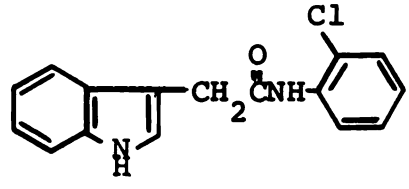
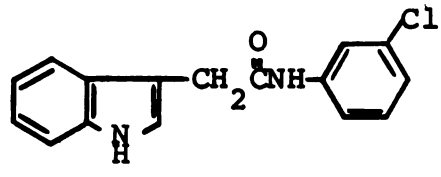
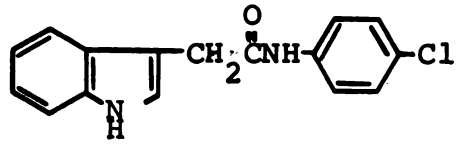
APPENDIX

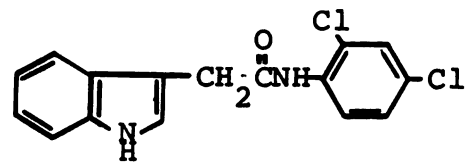
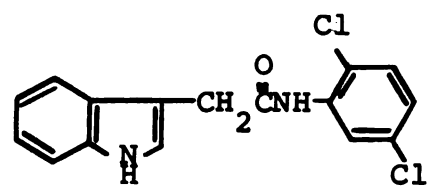
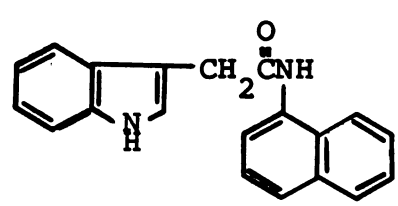
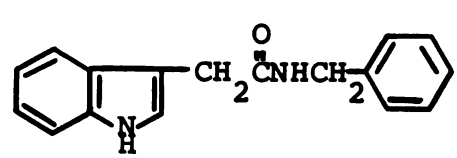
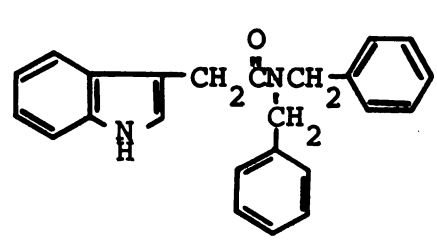
APPENDIX

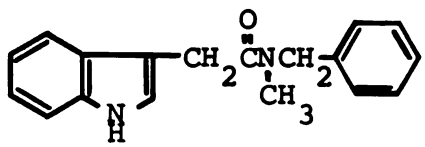
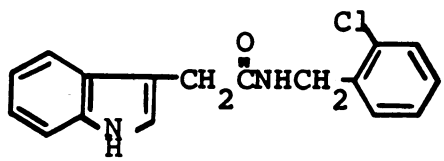
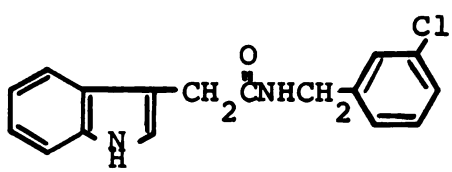
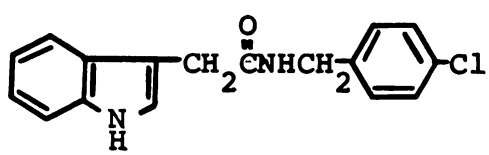
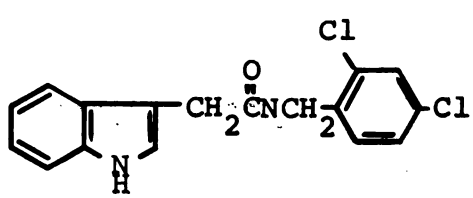
Chemical Name	Structure
Indol-3-ylacetic acid	
Indol-3-ylacetamide	
N-methylindol-3-yl-acetamide	
N,N-dimethylindol-3-yl-acetamide	
N-ethylindol-3-yl-acetamide	

Chemical Name	Structure
N,N-diethylindol-3-yl-acetamide	
N-(2-chloroethyl)-indol-3-ylacetamide	
N-propylindol-3-yl-acetamide	
N,N-dipropyl-indol-3-ylacetamide	
N-(3-chloropropyl)-indol-3-ylacetamide	

Chemical Name	Structure
N-isopropyl-indol-3-ylacetamide	
N,N-diisopropyl-indol-3-ylacetamide	
N-cyclohexyl-indol-3-ylacetamide	
N-dimethylamino-indol-3-ylacetamide-(indol-3-ylacetic acid-2,2-dimethylhydrazide)	
Indol-3-ylacetanilide	

Chemical Name	Structure
N,N-diphenyl-indol-3-ylacetamide	
N-methylindol-3-yl-acetanilide	
N-(2-chlorophenyl)-indol-3-ylacetamide	
N-(3-chlorophenyl)-indol-3-ylacetamide	
N-(4-chlorophenyl)-indol-3-ylacetamide	

Chemical Name	Structure
N-(2,4-dichlorophenyl)-indol-3-ylacetamide	
N-(2,5-dichlorophenyl)-indol-e-ylacetamide	
N-(1-naphthyl)-indol-3-ylacetamide	
N-benzylindol-3-yl-acetamide	
N,N-dibenzyl indol-3-ylacetamide	

Chemical Name	Structure
N-benzyl-N-methyl-indol-3-ylacetamide	
N-(2-chlorobenzyl)-indol-3-ylacetamide	
N-(3-chlorobenzyl)-indol-3-ylacetamide	
N-(4-chlorobenzyl)-indol-3-ylacetamide	
N-(2,4-dichlorobenzyl)-indol-3-ylacetamide	

Chemical Name	Structure
N-(3,4-dichlorobenzyl)-indol-3-ylacetamide