SYNTHESIS AND BIOLOGICAL ACTIVITY OF SEVERAL ETHYL 1-ACYLINDOLE-3-ACETATES

Thesis for the Degree of M. S.
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Roger William Ritzert

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By

ROGER WILLIAM RITZERT

AN ABSTRACT

Submitted to the School of Advanced Graduate Studies
Michigan State University of Agriculture and
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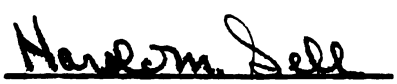
MASTER OF SCIENCE

Department of Biochemistry

1961

Approved 

Roger William Ritzert

ABSTRACT

Derivatives of indole-3-acetic acid (IAA), with the exception of ethyl indole-3-acetate (IAE), are generally equal to or less active than the parent compound. IAE is an exception in that greater activity than IAA has been demonstrated in several physiological systems. Several ethyl 1-acylindole-3-acetates were synthesized and the effects of substitution on biological activity were determined.

Equimolecular amounts of IAE and the appropriate acyl chloride were refluxed with benzene or toluene for 24 to 48 hours. The products were obtained by crystallization from various solvents. The following acyl groups were substituted in this manner: chloroacetyl, dichloroacetyl, 2-chloropropionyl, and 3-chloropropionyl. Acetyl-IAE was obtained by condensation of 1-acetylindole and ethyl diazoacetate. These derivatives, and 4-nitrobenzoyl and 4-aminobenzoyl-IAE obtained from another source, were assayed for their biological activity.

Comparative biological activity was determined in the promotion of parthenocarpic tomato ovary growth, bean petiole abscission, and Avena coleoptile straight growth. In the promotion of parthenocarpic growth of tomato ovaries, at 10^{-3} M in lanolin, dichloroacetyl-IAE was as active as IAE, and both were more active than IAA. Acetyl-IAE and

chloroacetyl-IAE were less active than IAE, but equal to IAA. The 2- and 3-chloropropionyl, 4-nitrobenzoyl, and 4-aminobenzoyl-IAE did not promote parthenocarpic growth of tomato ovaries. Dichloroacetyl-IAE was the most active compound in delaying bean petiole abscission. Acetyl, chloroacetyl, and 4-nitrobenzoyl-IAE were slightly more active than either IAE or IAA. However, 2- and 3-chloropropionyl, and 4-aminobenzoyl-IAE did not significantly delay petiole abscission. All of the IAE derivatives synthesized were active in the Avena coleoptile straight growth test over a wide concentration range. There was no marked effect due to substitution on the 1-indole position on biological activity of IAE as measured by the Avena straight growth assay.

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INTRODUCTION

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Auxins are defined as "compounds characterized by their capacity to induce elongation in shoot cells" (24). Auxins are those compounds which resemble the principal naturally occurring auxin, indole-3-acetic acid (IAA), in physiological action. It should be emphasized that cell elongation is not the only response observed. Auxins are also responsible for the control of cell differentiation, reproduction, abscission of leaves and fruit, and apical dominance. They are widely used in agriculture as herbicides, for fruit thinning, for control of pre-harvest drop, for setting of parthenocarpic fruit, for control of flowering, and for induction of rooting.

The majority of the auxins known are not structurally related to IAA. Many compounds structurally related to the common auxins have been tested for their ability to induce cell elongation, but few were as active as IAA. Of the several IAA derivatives assayed, only a few were substituted at the one position. From the many compounds tested for cell elongation properties, theories as to the structural requirements necessary for activity have been proposed.

This present study was initiated to determine if substitution on the 1-indole position of ethyl indole-3-acetate (IAE) alters the observed activity of the parent compound. The effects of different substituents were also of interest. IAE was used as the parent compound because

it is more active than IAA and because fewer complications in the acylation reaction were observed.

The problem was divided into two parts: (1) to find suitable methods for the acylation of ethyl indole-3-acetate on the 1-indole position and to determine the physical properties of the compounds synthesized; (2) to assay the compounds for their biological activity in three different physiological systems.

HISTORICAL

HISTORICAL

The first observation of the growth regulating properties of IAA was made by Kögl, *et al.* (13) after their isolation of the compound from human urine. It was observed that at low concentrations, IAA was capable of inducing cell elongation in *Avena* coleoptile sections. The first isolation of IAA from plant material, namely corn kernels, was accomplished by Haagen-Smit and co-workers (5), to suggest that IAA is one of the important naturally occurring growth hormones. Its presence in other plant tissues is now well documented (2). Although IAE has been shown to be more active than IAA in the tomato ovary test (19, 20) and in the *Avena* straight growth assay (18), it has been positively identified only in grape seeds and coconut milk (17).

Many synthetic compounds have been tested for their capacity to induce cell elongation. From the various compounds assayed, Koepfli, Thimann, and Went (12) postulated the following structural requirements for cell elongation activity:

(a) a ring system as nucleus

(b) a double bond in this ring

(c) a side chain

(d) a carboxyl group (or a structure readily converted to a carboxyl) on this side chain at least one carbon atom removed from the ring

(e) a particular space relationship between the ring and the carboxyl group.

Veldstra and Booij (26) condensed these five requirements into two. They proposed that in order to induce cell elongation, a compound must have the following structural characteristics:

(a) Basal ring system (non-polar part) with high interface activity.

(b) Carboxyl group (polar part) - in general a group of acidic character - in such a spatial position with respect to the ring system, that on absorption of the active molecule to a boundary (the non-polar part playing the most important role) this functional group will be situated as peripherally as possible.

Muir, Hansch, and co-workers (7, 8, 14, 15) have suggested that maximum cell elongation inducing properties are obtained when the positions ortho to the side chain (2- and 4-indole positions) are unsubstituted. Such an effect was observed for various phenoxyacetic acids and indole-3-acetic acids. This phenomenon has been called the "ortho effect." The same workers (8) have thus postulated a two-point attachment of auxin to the enzyme. However, this effect is not observed if 4-chloroindole-3-acetic acid is used, as it shows more activity than IAA (14).

It has also been observed (4, 10) that substitution on the phenylene nucleus often enhances activity in cell elongation assays and that substitution on the pyrrolene radical tends to inactivate the molecule for cell elongation properties. It has been shown that 1- and 2-methylindole-3-acetic

acids are inactive in the cell elongation assays (25). Sell, *et al.* (20) found that 1-benzoylindole-3-acetic acid is less active than IAA in the tomato fruit set assay.

Substitution for or alteration of the carboxyl group of IAA also appears to affect cell elongation activity. Nitsch and Nitsch (18) have shown that in the *Avena* straight growth test, IAE and indole-3-acetonitrile (IAN) are more active than IAA. Hamilton, *et al.* (6) observed a weak auxin effect when a tetrazole ring is substituted for the carboxyl group of IAA. Hofert and Sell (9) assayed several steryl esters of IAA for their capacity to set parthenocarpic tomato fruit and found them to be inactive.

Thimann (23) recently reported the biological activities of twenty indole compounds in the *Avena* straight growth test. He concluded that it is necessary to define the specific bioassay in which an auxin functions. Thimann also suggested that the empirical rules relating structure to activity in the aromatic series are also valid for the indole series, but that the requirements set forth in these theories are perhaps not as well described as they should be. It is further suggested that attempts to correlate structure with activity should perhaps include the physical effects of substitution on the nucleus.

This thesis reports the synthesis and biological activity of several ethyl 1-acylindole-3-acetates and the biological activity of two ethyl 1-aroylindole-3-acetates.

EXPERIMENTAL

EXPERIMENTAL

Synthesis of Compounds

The methods for the synthesis of the ethyl 1-acylindole-3-acetates and ethyl 1-triphenylmethylindole-3-acetate were chosen for their simplicity. The reaction used is the standard reaction of an acyl chloride with a secondary amine to give a tertiary amide, and in the case of the triphenylmethyl derivative, the reaction of triphenylmethyl chloride with a secondary amine to give a tertiary amine. In most cases, the reactions gave only fair yields. Crude oils were usually obtained initially and the desired crystalline product acquired from this crude mixture by use of various solvents. The reactions were made by refluxing in benzene or toluene for 24 to 48 hours using equimolecular amounts of IAE and the acyl chloride or triphenylmethyl chloride. Acetyl IAE was prepared from 1-acetylindole and ethyl diazoacetate.

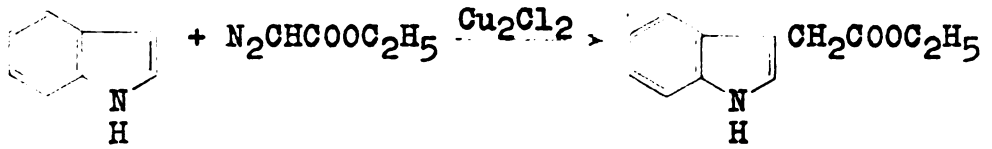
Two methods for the preparation of IAE were employed: one involved the condensation of indole with ethyl diazoacetate; and the other, the esterification of IAA with ethanol catalyzed by anhydrous hydrogen chloride.

The procedures are outlined below.

Ethyl Indole-3-acetate

Procedure A.

$$HCl \cdot NH_2CH_2COOC_2H_5 + NaNO_2 \longrightarrow N_2CHCOOC_2H_5$$



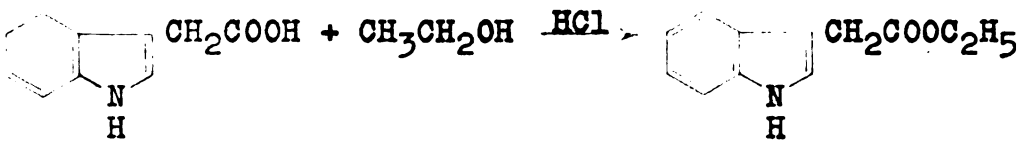
Ethyl diazoacetate (13). A cold solution of 210 g. (1.5 moles) of glycine ethyl ester hydrochloride and 1.05 g. of sodium acetate in 220 ml. of water was poured into a large beaker cooled in an ice bath. While this mixture was agitated with a motor stirrer, 157.5 g. (2.3 moles) of sodium nitrite in 220 ml. of water was added, followed by 125 ml. of ether. The bath was kept at 20°C. or lower by the addition of cracked ice throughout the remainder of the reaction. Twenty-five ml. of a 10% solution of sulfuric acid was slowly added and the mixture stirred until the reaction subsided (about 25 minutes). The complete mixture was then drawn into a large separatory funnel by application of a vacuum from a water aspirator at the top of the funnel. The aqueous layer was returned to the reaction vessel and the ether layer transferred to a smaller separatory funnel and washed immediately with a cold 10% solution of sodium carbonate.

The same cycle of operations was repeated using 100 ml. of ether and 25 ml. of a 10% solution of sulfuric acid. This was followed by a third cycle using 100 ml. of ether and 75 ml. of a 10% solution of sulfuric acid. The combined

ether solutions were then washed with a saturated sodium chloride solution. The ether solution was dried over anhydrous sodium sulfate, and the ether distilled off *in vacuo*. The last traces of ether were removed by passing a stream of dry nitrogen through the oil and 144.3 g. (84%) of a light yellow oil obtained. The product was stored in a dark bottle in the cold until used.

Ethyl Indole-3-acetate (16). To a solution of 46.8 g. (0.4 mole) of indole in 100 ml. of dry benzene in a three-necked flask was added 0.2 g. of cuprous chloride. The mixture was brought to reflux and the dropwise addition of 54.6 g. (0.48 mole) of ethyl diazoacetate in 320 ml. of dry benzene begun. The addition of ethyl diazoacetate was continued at such a rate that the evolution of nitrogen gas was constant. The mixture was refluxed for one hour after the addition was complete. The mixture was cooled, filtered, and the benzene removed under reduced pressure. The remaining oil was distilled *in vacuo*, and the product collected at 154-155°C. and 0.8 mm. pressure.[1] The slightly yellow oil weighed 37.5 g. (46%) and crystallized upon cooling.

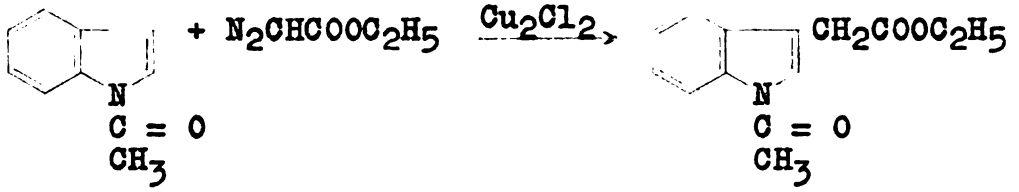
Procedure B (11).



1. Jackson (11) reports b.p. 180° and 2 mm. pressure.

To one liter of absolute ethanol saturated with 80 g. of anhydrous hydrogen chloride was added 60 g. (0.34 mole) of IAA. This mixture was allowed to stand at room temperature for five days after which the ethanol and hydrogen chloride were removed <u>in vacuo</u>. The crude oil that remained was distilled at 154-155°C. and 0.08 mm. pressure[1] to give 44 g. (64%) of IAE, which crystallized upon standing. The infrared spectrum showed $\lambda_{max.(u)}^{toluene}$ 2.90 (NH) and 5.76 (ester C = O).

Ethyl 1-Acetylindole-3-acetate



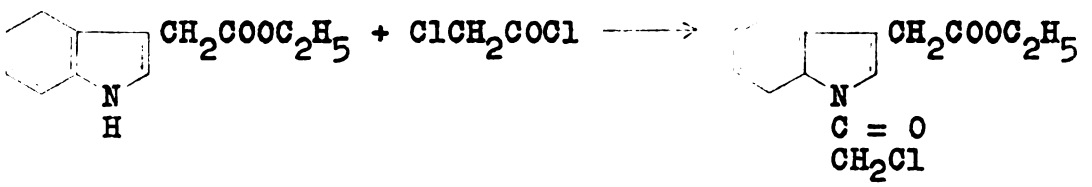
A solution of 15.9 g. (0.1 mole) of 1-acetylindole[2] in 30 ml. of dry benzene was brought to reflux. A small amount of cuprous chloride was added and the dropwise addition of 11.4 g. (0.1 mole) of ethyl diazoacetate in 60 ml. of dry benzene begun and continued at a rate necessary for a steady evolution of nitrogen gas. After three hours, the mixture was cooled, filtered, and the benzene removed <u>in vacuo</u>. The residual oil was distilled at 164-167°C. and 0.6 mm. pressure. The distillate upon redistillation gave 2.7 g. of a yellow oil of b.p. 142-147°C. and 0.3 mm. pressure. The product was dissolved in ethyl ether-hexane and upon cooling an orange oil soon separated out. The

1. Jackson (11) reports b.p. 180° and 2 mm. pressure.
2. Obtained from Regis Chemical Company.

supernatant mother liquor upon cooling at 0°C. gave 1 g. of colorless crystals melting at 90°C. The compound showed $\lambda_{max}^{toluene}$ (u) 5.81 (ester C = O), 5.93 (amide C = O), and no band near 2.90 (NH); $\lambda_{max}^{ethanol}$ (mu) (95%) 258 (log ϵ 4.174), and shoulder at 287-288 (log ϵ 3.498).

Anal. Calcd. for $C_{14}H_{15}NO_3$: C, 68.55; H, 6.16; N, 5.71. Found: C, 68.50; H, 6.12; N, 5.73.

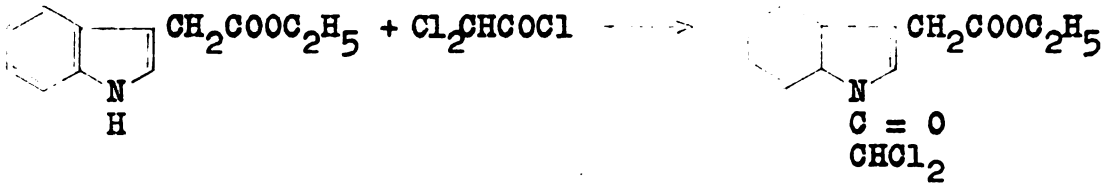
Ethyl 1-Chloroacetylindole-3-acetate



To a hot solution of 4.06 g. (0.02 mole) of IAE in 15 ml. of dry benzene was added dropwise a solution of 2.3 g. (0.02 mole) of chloracetyl chloride in 10 ml. of benzene. The mixture was refluxed for 24 hours, cooled, and the benzene removed <u>in vacuo</u>. The residue was dissolved in 95% ethanol, treated with Norite A, filtered, and the ethanol removed under reduced pressure. The residue was dissolved in 95% ethanol-ethyl ether and upon cooling gave colorless needles. Recrystallization from 95% ethanol gave 2.15 g. of colorless needles, m.p. 118-119°C. The absorption spectra showed $\lambda_{max}^{toluene}$ (u) 5.78 (ester C = O), 5.86 (amide C = O), and no band at about 2.90 (NH); $\lambda_{max}^{ethanol}$ (mu) (95%) 245 (log ϵ 4.090), 290 (log ϵ 3.738), and 300 (log ϵ 3.633).

Anal. Calcd. for $C_{14}H_{15}ClNO_3$: C, 60.11; H, 5.04; N, 5.00. Found: C, 59.66; H, 5.19; N, 4.87.

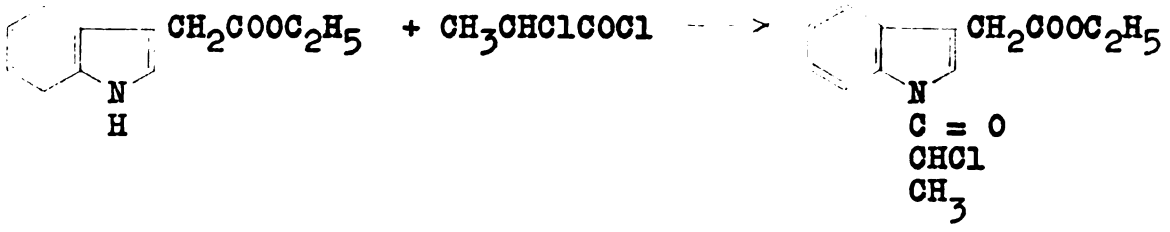
Ethyl 1-Dichloroacetylindole-3-acetate



A solution of 4.06 g. (0.02 mole) of IAE was dissolved in 25 ml. of dry toluene and the solution brought to reflux. The dropwise addition of 2.95 g. (0.02 mole) of dichloroacetyl chloride was begun and the mixture refluxed for 30 hours. The mixture was cooled and the toluene removed in vacuo. The residue was dissolved in 95% ethanol, treated with Norite A, and filtered. The product was precipitated by the addition of water, and cooling. Recrystallization from 95% ethanol gave 2.65 g. of colorless needles, m.p. 74°C. Absorption spectra showed $\lambda_{max\ (u)}^{toluene}$ 5.79 (ester C = 0), 5.88 (amide C = 0), and no band at about 2.90 (NH); $\lambda_{max\ (mu)}^{ethanol\ (95\%)}$ 250 (log ϵ 4.228), 291 (log ϵ 3.691), and 304 (log ϵ 3.733).

Anal. Calcd. for $C_{14}H_{13}Cl_2NO_3$: C, 53.52; H, 4.17; N, 4.45. Found: C, 53.91; H, 4.28; N, 4.32.

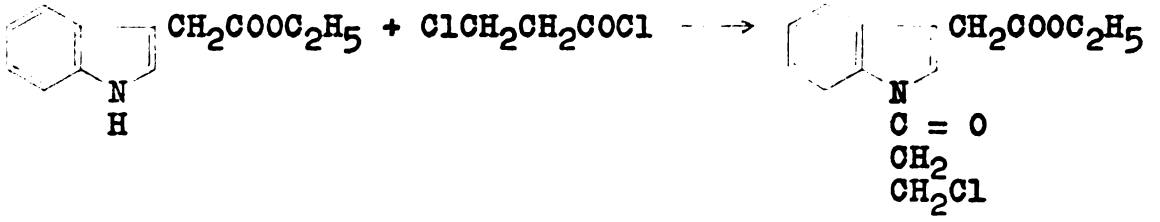
Ethyl 1-(2-Chloropropionyl)indole-3-acetate



A solution of 4.06 g. (0.02 mole) of IAE in 15 ml. of dry toluene was brought to reflux and the dropwise addition of 2.6 g. (0.02 mole) of 2-chloropropionyl chloride in 10 ml. of dry toluene begun. The mixture was refluxed for 48 hours, cooled, and the toluene removed <u>in vacuo</u>. The resulting oil was dissolved in ethanol, treated with Norite A, and filtered. The ethanol was removed under reduced pressure, the residue dissolved in hexane-ethanol, and the solution placed in the cold for three weeks. The desired product upon recrystallization from ethanol gave 1.04 g. of light yellow platelets, m.p. 63°C. The absorption spectra showed $\lambda_{max}^{toluene}$ (u) 5.75 (ester C = 0), 5.86 (amide C = 0), and no band at about 2.90 (NH); $\lambda_{max}^{ethanol(95\%)}$ (mu) 241 (log ϵ 4.421), 292 (log ϵ 3.954), and 301 (log ϵ 3.988).

<u>Anal</u>. Calcd. for $C_{15}H_{16}ClNO_3$: C, 61.33; H, 5.49; N, 4.76. Found: C, 61.21; H, 5.42; N, 4.77.

Ethyl 1-(3-Chloropropionyl)indole-3-acetate

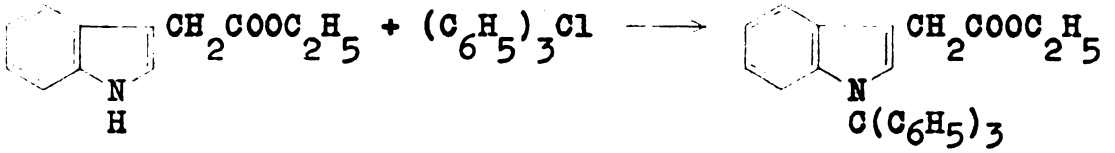


A solution of 4.06 g. (0.02 mole) of IAE in 15 ml. of dry toluene was brought to reflux and a solution of 2.6 g. (0.02 mole) of 3-chloropropionyl chloride in 10 ml. of toluene added dropwise. The mixture was refluxed for 48 hours, cooled, and the toluene removed <u>in vacuo</u>. The residue

was dissolved in ethanol, treated with Norite A, filtered, and the ethanol removed under reduced pressure. The residue was crystallized from ethanol-water at 0°C. The product upon recrystallization from ethanol gave 1.6 g. of colorless needles, m.p. 75°C. The absorption spectra showed $\lambda_{max\ (u)}^{toluene}$ 5.74 (ester C = O), 5.85 (amide C = O), and no band at about 2.90 (NH); $\lambda_{max\ (mu)}^{ethanol\ (95\%)}$ 248 (log ∈ 4.445), 266 (log ∈ 4.589), 292 (log ∈ 3.955), and 300 (log ∈ 3.964).

Anal. Calcd. for $C_{15}H_{16}ClNO_3$: C, 61.33; H, 5.49; N, 4.76. Found: C, 61.89; H, 5.28; N, 4.97.

Ethyl 1-Triphenylmethylindole-3-acetate



To a solution of 4.06 g. (0.02 mole) of IAE in 15 ml. of dry toluene was added 5.6 g. (0.02 mole) of triphenylmethyl chloride. The mixture was refluxed for 18 hours, cooled, and the toluene removed in vacuo. The residue was crystallized from ethyl acetate-ethanol at 0°C. Recrystallization of the product from ethyl acetate-ethanol gave 1.16 g. of colorless platelets, m.p. 179-180°C. The absorption spectra showed $\lambda_{max\ (u)}^{toluene}$ 5.75 (ester C = O), and no band at about 2.90 (NH); $\lambda_{max\ (mu)}^{ethanol\ (95\%)}$ 277 (log ∈ 3.853), 283 (log ∈ 3.881), and 294 (log ∈ 3.831).

Anal. Calcd. for $C_{31}H_{27}NO_2$: C, 83.56; H, 6.11; N, 3.14. Found: C, 83.28; H, 6.01; N, 3.46.

Characterization of Compounds

All melting points were obtained on the Fisher-Johns melting point block and are uncorrected. Carbon and hydrogen analyses were determined by Spang Microanalytical Laboratory of Ann Arbor, Michigan. Nitrogen analyses were made using the micro-Dumas method (22) or by Spang Laboratory.

Ultraviolet absorption spectra of the compounds, in 95% ethanol, were determined with the Beckman model DK-2 ratio-recording spectrophotometer using matched silica cells of one centimeter path length.

Infrared spectra of the compounds, in spectrograde toluene, were obtained with the Beckman model IR-5 double-beam recording spectrophotometer. A sodium chloride cell of 0.1 mm. path length was used for the sample and a variable path cell, adjusted to cancel out absorption by toluene, employed in the reference beam.

Biological Assays

Tomato Ovary Growth

Lanolin solutions. Known quantities of each compound were dissolved in 100 ml. of peroxide-free ethyl ether to give a solution of 1×10^{-3} molar concentration. One milliliter of this solution was mixed with one milliliter of lanolin in test tubes and the ether removed from the mixture by heating the tubes in a hot water bath. The solutions were stored in the cold until ready for use.

Plant material. Tomato plants (var. Michigan-Ohio hybrid) of comparable size and nutritional status were grown in the greenhouse. Three ovaries from the first flower cluster of each plant were emasculated 24 hours before anthesis and the remaining ovaries were removed from that cluster. Three replications were utilized for each treatment.

Application of lanolin solutions. Approximately 15 mg. of the lanolin solution of the appropriate compound was applied to each ovary and pure lanolin was used as a control. The diameter, in millimeters, of each ovary was measured four days after treatment, and this diameter was used as a measure of ovary growth. The mean of the three ovaries was used as the ovary diameter for one replication. The means were analyzed using the analysis-of-variance

method described by Snedecor (21). The means were further compared for significant difference using Duncan's (3) multiple range test.

Bean Petiole Abscission

Plant material. Bean plants (var. Contender) were grown in the greenhouse with four plants per pot, from which two of equal size were selected for the experiment. Four replications were used for each treatment. One primary leaf blade of each plant was removed when the first trifoliate leaf was beginning to unfold from the terminal bud, leaving about one centimeter of the petiole.

Method of treatment. The lanolin solutions of 1×10^{-3} M from the previous assay were used. The cut end of the petiole was covered with the lanolin solution immediately after debladding, and pure lanolin was used for the control. To determine when the petioles readily abscissed, they were tapped with a pencil every 12 hours on the upper surface, using approximately the same pressure for all petioles. The mean time for abscission to occur in each treatment was calculated. Using the lanolin control as 100%, the percent activity of the different treatments compared to control was determined.

Avena Straight Growth

Preparation of solutions. Sufficient quantities of each compound to give 200 ml. of 10^{-4} molar concentration were dissolved in one milliliter of Tween 20 and adjusted to volume. Aliquots were diluted to give solutions of 10^{-5}, 10^{-6}, 10^{-7}, and 10^{-8} molar concentrations. To 100 ml. of each concentration was added 3 ml. of phosphate-citrate buffer of pH 5.0 containing sufficient sucrose to give a 3% solution of sucrose upon dilution. The stock buffer solution was made accordingly: 11.97 g. of dibasic potassium phosphate, 6.80 g. of citric acid monohydrate, and 200 g. of sucrose were dissolved in water and adjusted to a volume of 200 ml. A buffer control containing 3% sucrose and a Tween 20 control with buffer using a 0.5% solution of Tween 20 for the 10^{-4} M concentrations and appropriate dilutions for the other concentrations.

Plant material. Brighton oats were submerged in distilled water in a suction flask and soaked under vacuum for two hours to remove the natural auxins. The supernatant water was discarded and the seeds placed on paper toweling over glass plates. The plates were placed in germinating dishes, to which a small amount of water had been added, and covered. The seeds were allowed to germinate in the dark at 25°C. After 24 hours, the seeds were exposed to two hours of red light to inhibit elongation

of the first internodes. After three days, coleoptiles of uniform length were selected. This and all subsequent steps were carried out under red light. Five millimeter sections were cut from the coleoptiles so as to exclude the apical 3 or 4 mm. The sections were floated on distilled water for one hour before treatment.

Method of treatment. Ten milliliters of solution containing the growth substance was placed in a 50-ml. flask, using three replications for each. Eight coleoptile sections were placed in the solution. The sections were allowed to elongate in the dark at 25°C. for 24 hours and the length of the sections in millimeters was measured. The mean values were expressed as percent of the initial 5 mm. length. The control was taken as an average of the growth observed for the Tween-buffer control at dilute concentrations of Tween 20. Inhibition with 0.5% Tween 20 was observed, but was not significant to warrant further correction of the results.

RESULTS AND DISCUSSION

RESULTS AND DISCUSSION

The melting points and results of the elemental analyses for the compounds are summarized in Table I. The molecular weights and ultraviolet absorption maxima are given in Table II.

In Figures 1, 2, and 3 are shown the infrared absorption spectra of the ethyl indole-3-acetate derivatives which were synthesized. The important absorption bands are summarized in Table III. That substitution has occurred at the 1-indole position is shown by the absence of the NH band at about 2.90u, which is the wavelength for the NH of IAE. Bellamy (1) reports the NH absorption for indole to be at 3491 $cm.^{-1}$ or 2.86u. For all of the compounds, the ester carbonyl band is present and is found between 5.74u and 5.81u. The ester carbonyl band is reported to be at 1750-1735 $cm.^{-1}$ or 5.71-5.76u. For those compounds which are acylated at the 1-indole position, the amide carbonyl band appears between 5.85u and 5.93u. Bellamy reports this band as being at 1690 $cm.^{-1}$ or 5.91u for those tertiary amides in which a phenyl group is substituted on the nitrogen atom.

The ability of these derivatives to induce the growth of parthenocarpic fruit was measured in the tomato ovary assay. The results of this assay are summarized in Table IV. A comparison of the mean values of ovary diameter shows that IAE and dichloroacetyl-IAE are more active in

Table I. Melting points and elemental analysis of indole compounds

Compound	Melting point, degrees, C.[1]	Calcd.			Found		
		C	H	N	C	H	N
Ethyl 1-Acetylindole-3-acetate	90	68.55	6.16	5.71	68.50	6.22	5.73
Ethyl 1-Chloroacetylindole-3-acetate	118-119	60.11	5.04	5.00	59.66	5.19	4.87
Ethyl 1-Dichloroacetylindole-3-acetate	74	53.52	4.17	4.47	53.91	4.28	4.32
Ethyl 1-(2-Chloropropionyl)indole-3-acetate	63	61.33	5.49	4.76	61.21	5.42	4.77
Ethyl 1-(3-Chloropropionyl)indole-3-acetate	75	61.33	5.49	4.76	61.89	5.28	4.97
Ethyl 1-Triphenylmethylindole-3-acetate	179-180	83.56	6.11	3.14	83.28	6.01	3.46

1. Melting points are uncorrected.

Table II. Ultraviolet absorption characteristics of indole compounds

Compound	Molecular weight	Absorption maxima[1]	
		λ in mu	Log ε
Ethyl 1-Acetylindole-3-acetate	245.27	287-288[2]	3.497
		258	4.174
Ethyl 1-Chloroacetylindole-3-acetate	279.72	245	4.090
		290	3.738
		300	3.633
Ethyl 1-Dichloroacetylindole-3-acetate	314.17	250	4.228
		291	3.691
		304	3.733
Ethyl 1-(2-Chloropropionyl)indole-3-acetate	293.75	241	4.421
		292	3.954
		301	3.988
Ethyl 1-(3-Chloropropionyl)indole-3-acetate	293.75	248	4.445
		266	4.589
		292	3.955
		300	3.964
Ethyl 1-Triphenylmethylindole-3-acetate	445.54	277	3.853
		283	3.881
		294	3.831

1. Ethanol (95%) was used as solvent.
2. Shoulder.

Figure 1

Infrared absorption spectra in toluene of ethyl indole-3-acetate (top), ethyl 1-chloroacetylindole-3-acetate (center), and ethyl 1-dichloroacetylindole-3-acetate.

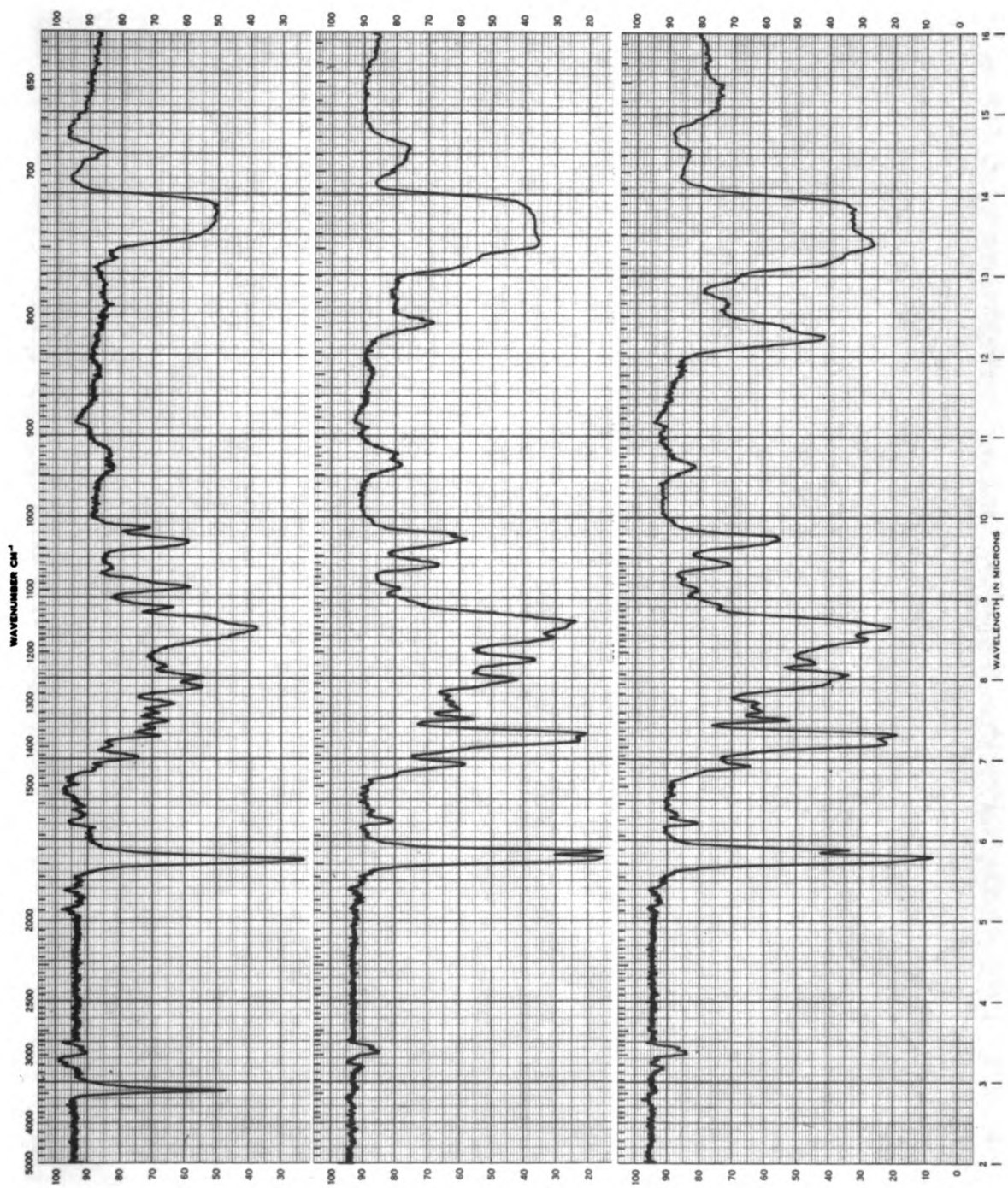

WAVENUMBER CM⁻¹
WAVELENGTH IN MICRONS

Figure 2

Infrared absorption spectra in toluene of ethyl 1-(2-chloropropionyl)indole-3-acetate (top), ethyl 1-(3-chloropropionyl)indole-3-acetate (center), and ethyl 1-triphenylmethyl-indole-3-acetate.

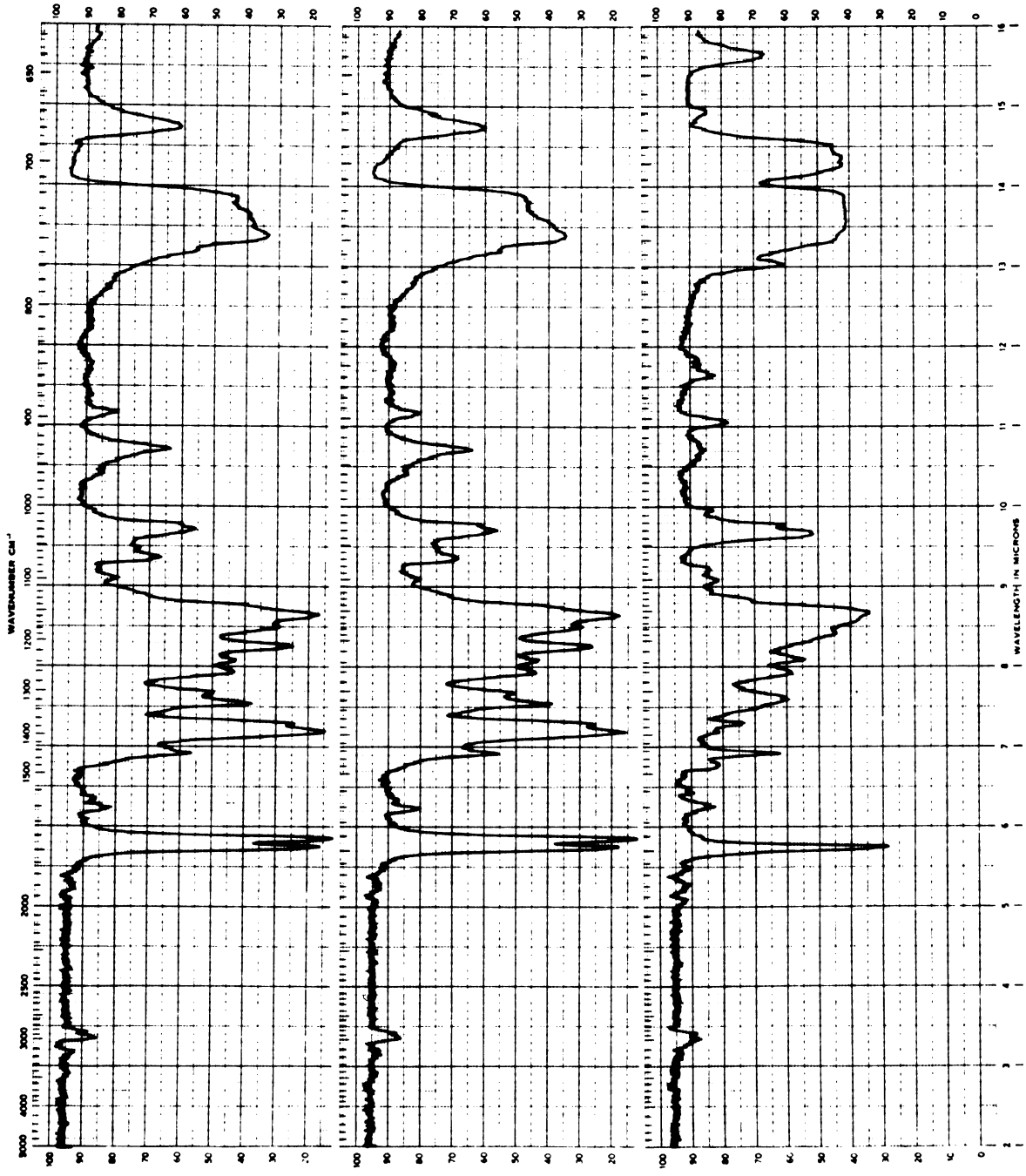

WAVENUMBER CM⁻¹
WAVELENGTH IN MICRONS

Figure 3

Infrared absorption spectrum in toluene
of ethyl 1-acetylindole-3-acetate.

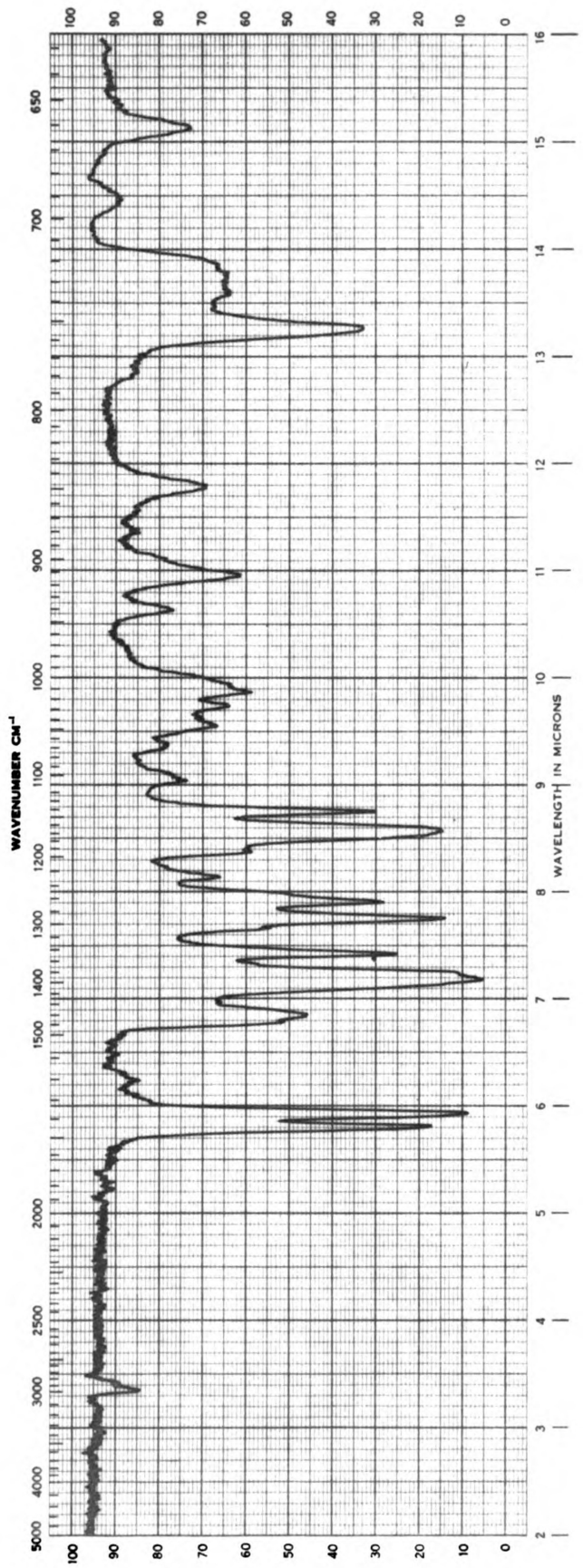

WAVENUMBER CM⁻¹
WAVELENGTH IN MICRONS

Table III. Infrared absorption characteristics of indole compounds

Compound	Absorption Maxima[1], λ in u		
	NH	Ester C=O	Amide C=O
Ethyl Indole-3-acetate	2.90	5.76	--
Ethyl 1-Acetylindole-3-acetate	--	5.81	5.93
Ethyl 1-Chloroacetylindole-3-acetate	--	5.78	5.86
Ethyl 1-Dichloroacetylindole-3-acetate	--	5.79	5.88
Ethyl 1-(2-Chloropropionyl)indole-3-acetate	--	5.75	5.86
Ethyl 1-(3-Chloropropionyl)indole-3-acetate	--	5.74	5.85
Ethyl 1-Triphenylmethyl indole-3-acetate	--	5.75	--

1. Spectro-grade toluene used as solvent.

this system than IAA. Acetyl-IAE and chloroacetyl-IAE possess the same order of activity as does IAA. However, 2- and 3-chloropropionyl-IAE, 4-nitrobenzoyl-IAE[3], and 4-aminobenzoyl-IAE[3] did not show ability to set parthenocarpic fruit. These compounds with larger substituent groups are perhaps inactive because they are not able to penetrate the ovary tissue.

In the bean petiole abscission assay, the capacity of the compounds to delay abscission of the debladed petiole was measured. The results of this assay are shown in Table IV. Dichloroacetyl-IAE was the most active compound in this assay. Acetyl-IAE, chloroacetyl-IAE, and 4-nitrobenzoyl-IAE were slightly more active than both IAA and IAE. However, 2- and 3-chloropropionyl-IAE, and 4-aminobenzoyl-IAE were not more active than IAA or IAE. In this system, IAE appears to be somewhat more active than IAA, as it is in the other assays.

The Avena straight growth test measures the capacity of the compounds to induce cell elongation. The coleoptile elongation in solutions of the various compounds is compared in Figure 4. The greater activity of IAE over IAA in the induction of cell elongation has been reported by Nitsch and Nitsch (18). All of the other derivatives assayed showed activity greater than that of IAA, and were as active as the parent compound, IAE. At 10^{-7}M, the two acyl derivatives with no halogen on the carbon adjacent to

3. Kindly supplied by Mrs. Richard Titus.

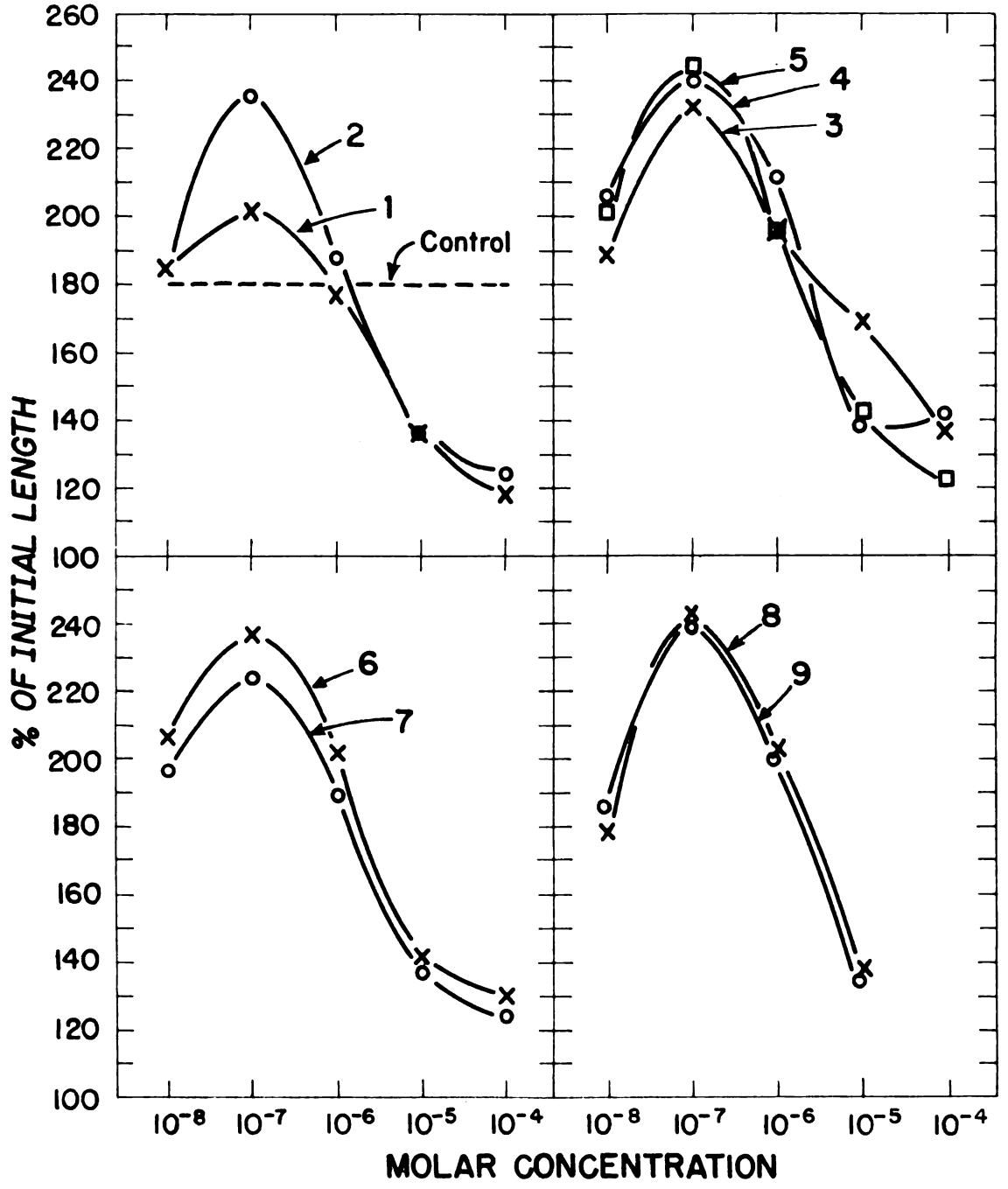
Table IV. Results of tomato ovary growth and bean petiole abscission assays

Compound, 1 x 10^{-3}M in lanolin	Tomato ovary growth		Bean petiole abscission
	Mean[1] values	Comparison[2] of means	Activity[3] as % of control
None	3.3	f	100
4-Chlorophenoxy acetic acid	9.2	a	---
Indole-3-acetic acid	5.3	de	123
Ethyl Indole-3-acetate	7.7	ab	138
Ethyl 1-Acetylindole-3-acetate	5.7	cd	162
Ethyl 1-Chloroacetylindole-3-acetate	5.3	de	169
Ethyl 1-Dichloroacetylindole-3-acetate	7.2	bc	200
Ethyl 1-(2-Chloropropionyl)indole-3-acetate	3.8	ef	123
Ethyl 1-(3-Chloropropionyl)indole-3-acetate	3.7	ef	115
Ethyl 1-(4-Nitrobenzoyl)indole-3-acetate	3.7	ef	154
Ethyl 1-(4-Aminobenzoyl)indole-3-acetate	3.3	f	123

1. Diameter of ovary in millimeters measured four days after treatment.
2. Means followed by same letter or letters not significantly different at the 5% level.
3. Time required for abscission of debladed petiole.

Figure 4

Growth curves of Avena coleoptile sections in various treating solutions: (1) IAA, (2) IAE, (3) acetyl-IAE, (4) chloroacetyl-IAE, (5) dichloroacetyl-IAE, (6) 2-chloropropionyl-IAE, (7) 3-chloropropionyl-IAE, (8) 4-nitrobenzoyl-IAE, and (9) 4-aminobenzoyl-IAE. Control represents the Tween 20 control as described in the text.


260
240
220
200
180
160
140
120
100
% OF INITIAL LENGTH
Control
1
2
3
4
5
6
7
8
9
10⁻⁸
10⁻⁷
10⁻⁶
10⁻⁵
10⁻⁴
MOLAR CONCENTRATION

the amide carbonyl were slightly less active than those with a halogen at this position. This is demonstrated by acetyl-IAE and 3-chloropropionyl-IAE. However, such an effect is not true with the two benzoyl derivatives.

The biological activity of these indole derivatives indicates that substitution at the 1-position does not affect the activity observed with IAE in the Avena straight growth test. However, further experiments must be made to determine whether the molecule remains intact during the growth period. Further work is also necessary to decide if other substituents at the 1-indole position may increase or decrease the auxin activity observed with the cell elongation assay.

It is apparent from the three assays that substitution on the 1-indole position does not alter the activity observed with IAE, but that the alteration of activity is due to the specific group substituted.

SUMMARY

SUMMARY

The methods of synthesis and the biological activity for several ethyl 1-acylindole-3-acetates, and the synthesis of ethyl 1-triphenylmethylindole-3-acetate are given. The following acyl derivatives were prepared:

Ethyl 1-Acetylindole-3-acetate
Ethyl 1-Chloroacetylindole-3-acetate
Ethyl 1-Dichloroacetylindole-3-acetate
Ethyl 1-(2-Chloropropionyl)indole-3-acetate
Ethyl 1-(3-Chloropropionyl)indole-3-acetate

The melting points, and the ultraviolet and infrared absorption spectra were determined for the compounds. Results of the elemental analysis for carbon, hydrogen, and nitrogen are also reported.

In addition to the above compounds, ethyl 1-(4-nitrobenzoyl)indole-3-acetate and ethyl 1-(4-aminobenzoyl)-indole-3-acetate were assayed for biological activity. The acetyl, chloroacetyl, and dichloroacetyl derivatives were active in the tomato ovary growth and the bean petiole abscission assays, and the nitrobenzoyl derivative showed activity in the latter. All of the compounds showed activity in the *Avena* straight growth test, and were as active as ethyl indole-3-acetate.

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