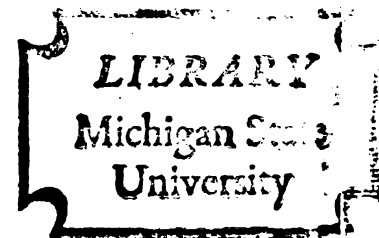SYNTHESIS AND PHYSIOLOGICAL
ACTIVITY OF SEVERAL
1-SUBSTITUTED INDOLE-3-ACETIC
ACIDS AND ESTERS

Thesis for the Degree of Ph. D.
MICHIGAN STATE UNIVERSITY
Robert Louis Franklin
1967

This is to certify that the

thesis entitled

SYNTHESIS AND PHYSIOLOGICAL
ACTIVITY OF SEVERAL
1-SUBSTITUTED INDOLE-3-ACETIC
ACIDS AND ESTERS
presented by

Robert Louis Franklin

has been accepted towards fulfillment
of the requirements for

__Ph.D.__ degree in _Biochemis_try

Harold M. Sell
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ABSTRACT

SYNTHESIS AND PHYSIOLOGICAL ACTIVITY OF SEVERAL
1-SUBSTITUTED INDOLE-3-ACETIC ACIDS AND ESTERS

by Robert Louis Franklin

Much scientific activity has followed the isolation
of indole-3-acetic acid and the discovery of the response
caused by this auxin in plants. Current research is attempt-
ing to discover the aspects of this and similar structures
which may be responsible for biological activity.

The present investigation was undertaken to study the
effect on plant growth response when substituents are attached
to the nitrogen atom of indole-3-acetic acid. The compounds
possessed a range of steric properties and lipophilicities as
well as a range of electronic effects. Each of the following
compounds was synthesized and biologically assayed:

1-methylindole-3-acetic acid
1-ethylindole-3-acetic acid
1-n-propylindole-3-acetic acid
1-iso-propylindole-3-acetic acid
1-n-butylindole-3-acetic acid
1-iso-butylindole-3-acetic acid
1-sec-butylindole-3-acetic acid
1-tert-butylindole-3-acetic acid
1-n-pentylindole-3-acetic acid
1-n-decylindole-3-acetic acid

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1-n-octadecylindole-3-acetic acid

ethyl 1-p-benzylindole-3-acetate

ethyl 1-p-chlorobenzylindole-3-acetate

ethyl 1-p-bromobenzylindole-3-acetate

ethyl 1-p-methylbenzylindole-3-acetate

ethyl 1-p-methoxybenzylindole-3-acetate

ethyl 1-p-nitrobenzylindole-3-acetate

methyl 1-β-D-glucopyranosylindole-3-acetate

Ultraviolet and infrared spectra were used to supplement ele-mental analysis in the characterization of the preceding com-pounds.

The synthesis and characterization of 51 other indole related compounds and their intermediates are also reported.

All of the alkyl substituted compounds had a level of activity relative to indole-3-acetic acid of less than 10% in four biological assays. These were: _Avena_ straight growth, buckwheat root inhibition, bean petiole abscission, and tomato ovary growth assays. However, in the cucumber curva-ture assay, 1-_iso_-propylindole-3-acetic acid was outstanding in its activity. The physiological response was approximately equal to that of indole-3-acetic acid, but was slower in appearance and much more persistent. A qualitative correla-tion existed between the _Avena_ activity of the acids substi-tuted with lower alkyl groups and the Taft E_s values, indicat-ing that steric factors are important in decreasing the activity of these derivatives.

A methyl group attached to the nitrogen atom lowers the activity normally associated with indole-3-acetic acid to approximately 10%, but larger groups gradually reduce the

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activity to the point of considerable inhibition. Thus, tert-butylindole-3-acetic acid at 10^{-4} M concentration was a strong inhibitor of growth in the Avena straight growth assay.

Several of the substituted benzyl esters had activity approaching that of indole-3-acetic acid in both the Avena and bean petiole abscission assays. Other tests involving intact plants did not respond well to these derivatives, an indication that they are not easily transported through an intact membrane.

The benzyl derivatives substituted with halogens showed a qualitative correlation between the Hammett σp values and the activity in both Avena straight growth and in buckwheat root inhibition. Factors other than electronic effects may be involved in the response of the different substituted benzyl esters as no obvious correlation could be made between activity and the σp value of the substituent.

Methyl 1-β-D-glucopyranosylindole-3-acetate had essentially no activity in any test as would be expected of a hydrophilic compound. The low activity is good evidence that this N-glucoside is not degraded in any of the plant systems investigated.

SYNTHESIS AND PHYSIOLOGICAL ACTIVITY OF SEVERAL

1-SUBSTITUTED INDOLE-3-ACETIC ACIDS AND ESTERS

By

Robert Louis Franklin

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Biochemistry

1967

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ACKNOWLEDGMENTS

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INTRODUCTION

In higher plants indole-3-acetic acid exerts control over a variety of essential functions such as flowering, apical dominance, fruit-setting, and root extension. Obviously, the existence of indole-3-acetic acid is of great importance in the coordination of plant growth. There exists a pressing need to learn the mode of action of plant growth regulators, both natural and synthetic, before further advances can be made in the intelligent use of these substances for controlling the size and yield of plants. The control of plant growth presents man with two contradictory challenges: first, plant growth must be encouraged to meet the demands of the rapidly expanding population; and second, plants that interfere with beneficial plants must be selectively destroyed. Therefore, both growth-stimulating and growth-inhibiting compounds are of importance. Moreover, the problem is also of interest in understanding the mechanism by which a chemical signal or hormone can coordinate and control diverse cellular functions. Ramifications of the solution to this problem extend throughout biochemical systems and encompass vital functions not well understood in biology and medicine (17, 28, 111).

In animal systems, a given hormone usually acts on a specific target organ; however, in plants, a hormone may

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elicit responses in a number of different organs. Consider-
able interplay exists between the known plant regulators as
they often exhibit synergism in their action (107). Such
phenomena complicate the study of plant hormones and neces-
sitate the use of several different bioassays in an attempt
to isolate the diverse responses in cellular activity.

While indole-3-acetic acid may not be the chemical
entity that functions at each site which responds to the
stimulus of this and related substances, the fact remains
that a multitude of biological responses can be elicited by
these compounds. Therefore, as one of the compounds most
deeply implicated in growth responses, indole-3-acetic acid
is a worthy candidate for chemical modification and observa-
tion of the changes wrought by this alteration. Thus, the
quest continues for new compounds which are more active than
the parent acid as well as those which will open new avenues
of research into the mechanism by which molecules may inter-
act intermolecularly or intramolecularly and with subcellu-
lar organelles.

Studies by Ritzert, Sell and Bukovac (81) have shown
that ethyl indole-3-acetate bearing a para-substituted benzoyl
group attached to the nitrogen has a reasonably high degree
of activity in tomato fruit-set and _Avena_ straight growth
assays. However, the question remains unanswered as to
whether this compound acted _per se_ or was degraded to an
active compound.

Although the benzyl radical is similar in steric

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requirement to the benzoyl radical, the latter is more susceptible to hydrolysis. In this work, substituted benzyl derivatives of ethyl indole-3-acetate were made by a known synthetic route (65) which would yield a compound approximately isosteric with the benzoyl analog. Porter and Thimann's (75, 76) hypothesis that the degree of auxin activity is proportional to the extent of partially positive charge on the indolic nitrogen, leads one to expect that benzyl derivatives would not exhibit as high a degree of activity as the aroyl compounds of Ritzert et al. (81). The activity of the 1-aroyl derivatives is enhanced by the additional electron withdrawing effect of the amide carbonyl. An investigation was made to determine whether or not benzyl compounds have significant activity and whether or not such activity could be correlated with the Hammett sigma constants of the substituents attached to the benzyl ring.

Hansch et al. (27) indicated a correlation of lipophilicity with activity in both plant growth regulators and in derivatives of the antibiotic chloromycetin. Mitchell and Linder (67) demonstrated that α-methoxylation and N-acetylation enhance absorption and translocation of the derivatives in comparison to indole-3-acetic acid. Alpha-methylation of indole-3-acetic acid was shown to enhance the fruit-setting activity of tomato plants (9).

Stowe and his coworkers (72, 94, 95, 96) demonstrated a growth response which can be attributed to long-chain fatty acid esters and related compounds. The response is

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most obvious in pea plants and is usually seen as a synergism involving the lipid as well as indole-3-acetic acid and gibberellic acid. These considerations as well as the observation of Baskakov and Mel'nikov (4) that 1-ethyl and 1-propyl indole-3-acetic acids are active in bean root growth add impetus to the hypothesis that 1-alkyl derivatives of indole-3-acetic acid are worthy of further study.

A series of 1-alkyl derivatives of indole-3-acetic acid were prepared which exhibit a range of steric requirement as well as a range of lipophilicity while maintaining a fairly constant electronic effect. These analogs allow an evaluation of the effect of steric bulk and lipophilicity of the group on activity unimpeded by electronic considerations.

Diametrically opposed in solubility to the lipophilic alkyl groups lies the hydrophilic glucosyl moiety. Therefore, the methyl ester of 1-β-D-glucopyranosylindole-3-acetic acid was synthesized and analyzed.

The series of 1-derivatives of indole-3-acetic acid in this investigation exhibit a span of solubility ranging from the highly lipophilic to the highly hydrophilic. The range of steric requirement encompasses carbon chains from one through eighteen atoms and includes two different ring systems. Electronic variations were induced in the indole ring by substituting the benzyl moiety with groups ranging in electronic effects from nitro to methoxy. An attempt was made to explain the observed biological effects of these derivatives with their structure.

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In addition to the physiological considerations and
their relationship to structure, part of this work was con-
cerned with synthesis. A novel route for the synthesis of
1-substituted indole-3-acetic acids is reported which
involves a sequence of known syntheses. Several different
pathways were investigated for the synthesis of similar com-
pounds. The use of dichlorodicyanobenzoquinone was found to
be generally favored over chloranil for aromatization of the
indoline nucleus. The synthesis of glycosyl indole compounds
is of interest as a possible model for other biochemically
important indole analogs.

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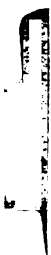
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HISTORICAL

Indole-3-acetic acid was first considered as a constituent of higher plants in 1909 (31) when Herter suggested its presence. It remained for Haagen-Smit and coworkers (23) to isolate and characterize the crystalline compound from immature Zea Mays kernels in 1946. However, Kögl, Haagen-Smit and Erxleben (50) had shown the importance of the acid in plant growth in 1934, when they isolated this crystalline material from human urine. The isolated indole-3-acetic acid was then shown to cause cell elongation in Avena coleoptiles.

Many instances of the occurrence of indole-3-acetic acid in higher plants have been reported (7). It is significant that evidence for its presence is almost always based on chromatographic data and bioassays.

Indole compounds, that can be degraded to indole-3-acetic acid, have also been reported. In 1944, Larsen (55) reported a compound that was tentatively identified as indole-3-acetaldehyde. Proof of this tentative structural proposal was later presented by the same investigator (56) from evidence gathered through comparison studies with authentic indole-3-acetaldehyde. This neutral growth substance was isolated from etiolated epicotyls of beans, peas, and sunflower plants.

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In 1953, Henbest, Jones, and Smith (30) isolated a substance that exhibited the same infrared spectrum as did authentic indole-3-acetonitrile. Both the aldehyde and the nitrile probably owe their activity to their degradation to indole-3-acetic acid in vivo (54, 102, 122).

Acidic analogs of indole-3-acetic acid have also been reported. These include indole-3-carboxylic acid, which has been shown to be present in plants such as pea, tomato, and Brassicae (18). Linder and coworkers (59) presented tentative evidence for the presence of β-indole-3-propionic acid in white cabbage and Brussel sprouts. Potato tubers during the dormancy period have been shown to contain ɣ-indole-3-butyric acid (8).

Recently, Zenk (127) has reported the isolation and characterization of a carboxy-bound glucose derivative of indole-3-acetic acid. Andreae and Good (1) found a similar compound linked through the indole-3-acetic acid carbonyl to the amino group of aspartic acid. Still more complex derivatives have been isolated from mature corn kernels (Zea Mays) by Bandurski and coworkers (25, 53).

The occurrence pattern of the glucose derivative and of the aspartate conjugate is such as to suggest an evolutionary pattern of development of the systems responsible for their synthesis (127). Some plants have the ability to produce both compounds. The physiological significance of these conjugates is not clear.

While no 1-substituted derivatives of indole-3-acetic

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acid have been reported as occurring naturally in plants,
several examples of synthetic derivatives showing signifi-
cant activity are found in the literature (81, 86). The
degree of activity, absorption, or translocalizability
sometimes equals or exceeds that of the parent acid.
Ritzert et al. (81) reported the synthesis and activity of
several ethyl 1-acylindole-3-acetates some of which had
activity greater than the parent acid. Alpha-methoxylation
and N-acetylation was shown by Mitchell and Linder (67) to
enhance absorption and translocation. However, activity
remained at approximately the same level as indole-3-acetic
acid.

Several workers (76, 107) have reported that nitrogen
methylation of indole-3-acetic acid greatly decreases its
activity. Porter and Thimann (76) indicate that the activity
is 13% that of indole-3-acetic acid. 1-Ethyl- and 1-propyl-
indole-3-acetic acid were synthesized and tested in a bean
root growth assay by Baskakov and Mel'nikov (3). They
reported enhancement of root growth by these compounds.

SYNTHESIS OF 1-SUBSTITUTED INDOLE DERIVATIVES

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SYNTHESIS OF 1-SUBSTITUTED INDOLE DERIVATIVES

Indole derivatives may be synthesized from a variety of precursors (82). Equally numerous are the synthetic approaches to indole compounds. However, reported syntheses of 1-substituted indoles are somewhat less numerous.

The Fischer indole synthesis technique is a classical method for most synthetic attempts at indole compounds. This route involves closure of the pyrrole ring and allows for a variety of substituents including those attached to the nitrogen. The starting materials are phenylhydrazine or a substituted phenylhydrazine (i) and a carbonyl compound (ii) with carbon atoms arranged in such a manner as to allow them to be incorporated into the nascent indole nucleus at positions 2 and 3.

$$(R''') H \quad NH-NH_2 \quad + \quad \begin{array}{c} CH_2-R'' \\ | \\ C \\ // \quad \backslash \\ O \quad R' \end{array} \quad \longrightarrow \quad (R''')H \quad N-N \quad \begin{array}{c} CH_2-R'' \\ | \\ C-R' \\ // \end{array} \quad \longrightarrow \quad (R''')H$$

(i)　　　　　(ii)　　　　　(iii)　　　　　(iv)
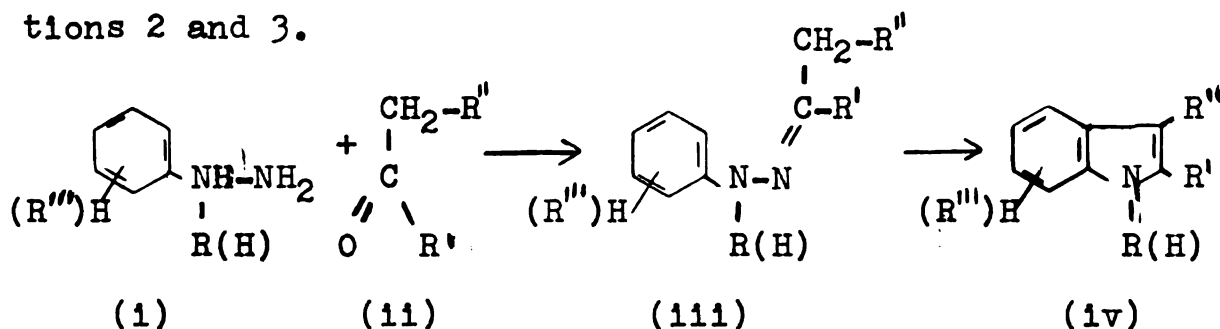
After formation of the intermediate arylhydrazone (iii), the ring is closed by treatment with an acid reagent such as zinc chloride or sulfuric acid. Recently pyrophosphate ester has been investigated as a catalyst (41). Although Fischer fused an excess of zinc chloride with the

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reagents, the procedure can be improved by the use of a high boiling solvent such as methylnaphtalene and a small amount of the catalyst (2). While the technique has wide applicability, the simplest case involving acetaldehyde phenylhydrazone does not give the expected indole. Activated ketones such as pyruvic acid react very well (82).

The limitations of the reaction are few but real. For the preparation of 1-substituted indoles it is necessary to prepare the corresponding phenylhydrazine. The possibility of steric hindrance caused by the substituent must also be considered. Assymetrically substituted phenylhydrazones offer the additional complication of two modes of ring closure:

(v)   (vi)   (vii)
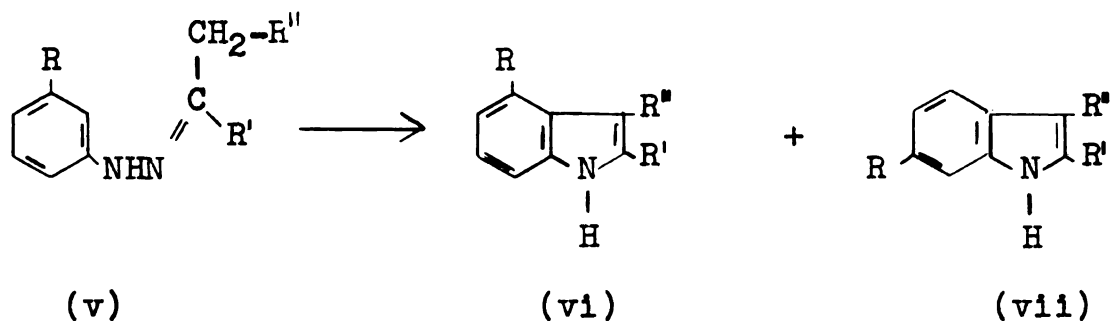
Another route to 1-substituted indoles is the Bischler synthesis which Julia and coworkers have investigated extensively (38, 39). An aniline (viii) substituted on the nitrogen is reacted with an α-haloketone (ix) in a two step

(viii)   (ix)   (x)   (xi)

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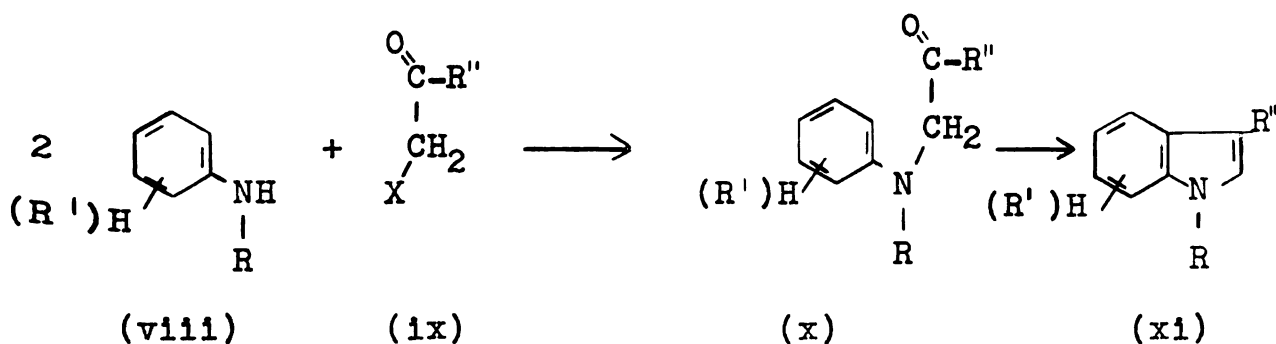
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reaction. The second step is often enhanced by using an acid reagent such an anhydrous zinc chloride. As with the Fischer method, the intermediates may be difficult to prepare. Another disadvantage of the Bischler method is the uneconomical use of an additional mole of the aniline to trap the evolved hydrogen halide. Nevertheless, the technique has the advantage of allowing for attachment of both 1- and 3-substituents simultaneously. By using ethyl-γ-bromoacetoacetate (xiii) it is possible to make 1-substituted ethyl esters of indole-3-acetic acid (xv) without careful purification of the intermediates (xiv) (40).
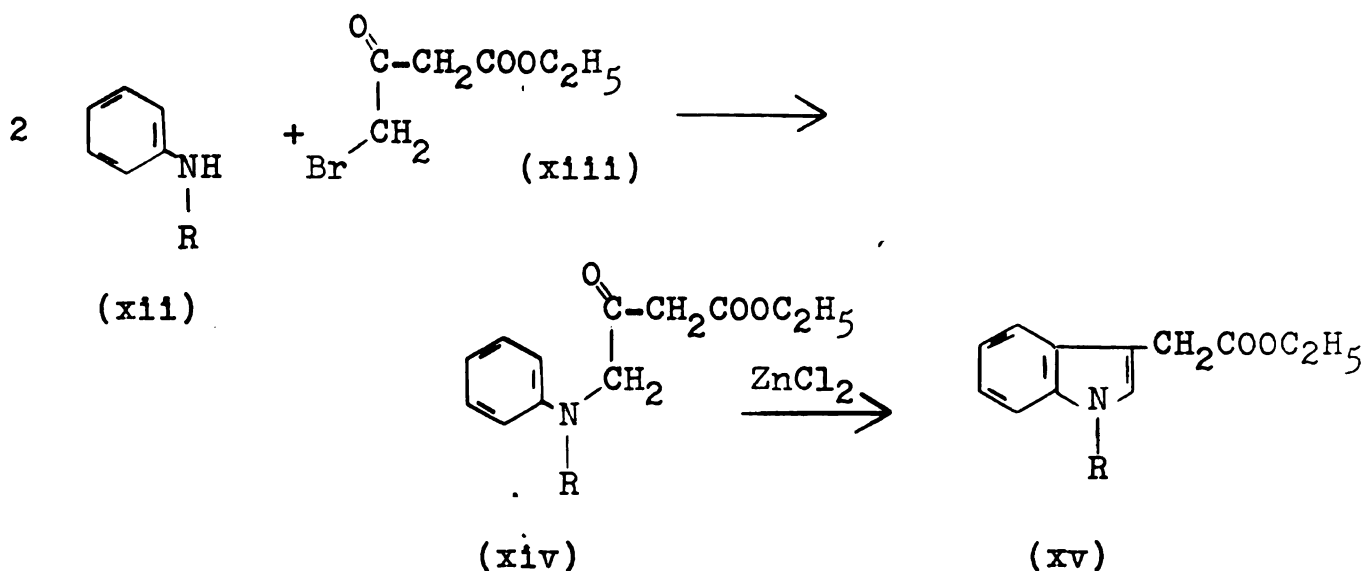
Several other synthetic procedures are available for providing indoles with 1-substituents. Some of these result in products which have an oxindole or isatin nucleus while others yield compounds with altered phenyl rings. In the Nenitzescu synthesis (5, 6, 15), benzoquinone (xvi) is treated with an N-substituted unsaturated-γ-amino ester (xvii). By this route a series of very important chemicals

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(xvi)   (xvii)   (xviii)
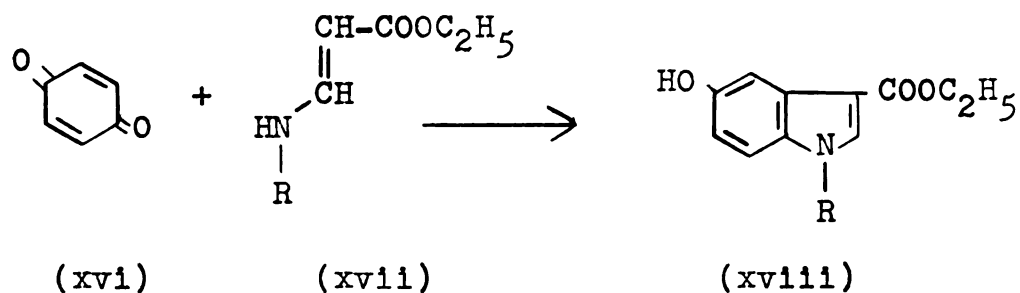
Alkylated anilines (xix) and "glyoxol bisulfite" (xx)
yield products hydrolyzable to 1-substituted oxindoles (xxi).

(xix)   (xx)   (xxi)
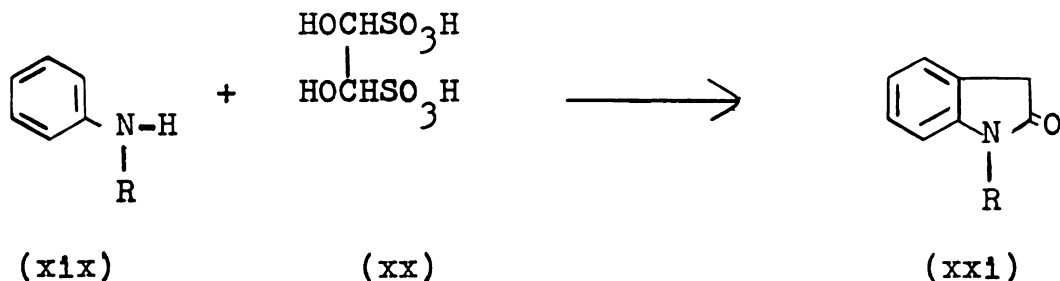
A versatile synthetic route which is particularly
useful for either 4-substituted or 4,5-disubstituted indoles
has been reported by Cornforth and coworkers (13). The
synthetic intermediates, 4-keto-4,5,6,7-tetrahydroindoles
(xxii), can be prepared by the reaction of 1,2-cyclohex-
anediones with α-haloketones followed by cyclization with
ammonia or primary amines (57).

(xxii)   (xxiii)
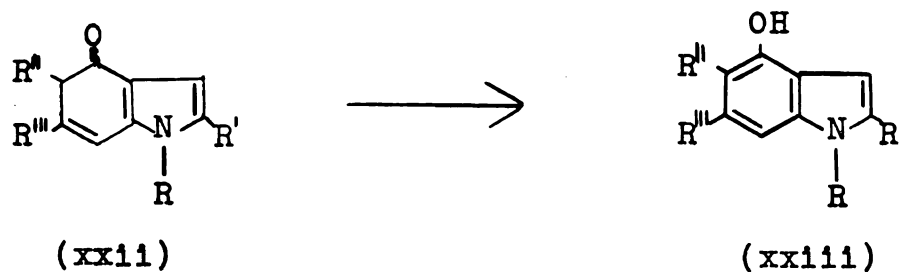
The product of the cyclization (xxii) can be converted

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to various other derivatives by manipulation of the groups at positions 4 and 5. Final aromatization of the ring to produce compound (xxiii) is best accomplished with 10% palladium on charcoal in refluxing cumene (13). Dehydrogenation with dichlorodicyanobenzoquinone is not successful unless the 5-position bears a hydroxymethylene group (80). Depending on whether ammonia or a primary amine is used in the ring closure step, 4-ketotetrahydroindoles with the ring nitrogen either free or alkylated, respectively, are obtained.

Direct alkylation is often employed to afford the substituted indoles (33, 82, 125). However, it is necessary to make an intermediate N-metal salt in order to get the desired nitrogen substitution. Reaction of indole with an alkyl halide gives a variety of carbon alkylations in addition to nitrogen alkylation (82). Excess methyl iodide yields tetramethylindolenium iodide (xxiv) when it is reacted with indole (82).

$$\text{(structure: indolenium ring with } CH_3, CH_3, CH_3 \text{ substituents; } N\text{-}CH_3; \ I^-\text{)}$$
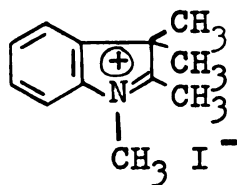
(xxiv)

Several partially methylated intermediates have been characterized which react further to yield the tetramethyl derivative.

To minimize these complications, alkylations are

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usually carried out in basic solution in the presence of
sodium or potassium (126). Other cations have been employed
including lithum and tetramethylammonium cations (57). How-
ever, with direct alkylation, liquid ammonia is often employed
as the solvent and so necessitates cryogenic apparatus. The
product from such a reaction is often obtained in good yield,
but suffers from contamination by unreacted starting material
which is not always easy to remove (71, 89). Prolonged
refluxing with sodium is sometimes used to effect separation
of unreacted starting material (33). Unfortunately, groups
such as iso-propyl and tert-butyl with a large steric require-
ment have not been attached by this technique even though
repeated attempts have been made (126).

Reactive halides such as benzyl halides will react
with the N-sodium salt of indole compounds in dimethylformam-
ide (65) solution. However, since this reaction is incomplete,
it requires careful fractionation of the products. It is very
versatile and numerous derivatives have been reported (60, 61,
62, 63) including many 1-acyl compounds.

Still another technique for preparing 1-substituted
indoles involves using a substituted indoline as an inter-
mediate (97, 98). A distinct advantage of this procedure is
the possibility of removing the unaltered indoline from the
product. Acetic anhydride or benzene sulfonyl chloride
(Hinsberg's reagent) may be employed to reduce the basicity
of indoline through reaction to yield the amide. The former
reagent has been used in a similar synthesis of N-methyl,

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N-__tert__-butyl aniline (126). The substituted indoline can
then be extracted from the amide with acid. Chloranil (97,
98) or dichlorodicyanobenzoquinone (78) can be used to
aromatize the substituted indoline ring.

The Madelung-Verley synthesis involving another ring
closure can also yield 1-substituted indoles (xxiii) (58,
82, 118). A basic condensing agent such as potassium __tert__-
butoxide is employed to cyclize the substituted aniline
(xxv).

$$\text{(xxv)} \xrightarrow{\text{t-Bu-O-K}} \text{(xxvi)}$$

(xxv)   (xxvi)
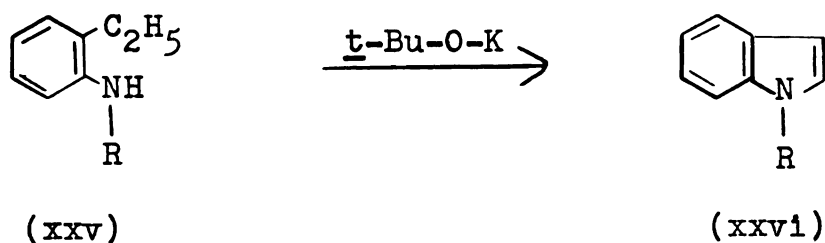
Other less useful routes to N-alkylated products
exist. For example isogramine (xxvii) can be made by con-
trolling carefully the conditions of the Mannich reaction
(99) in which gramine (xxviii) is usually produced.

(xxvii)   (xxviii)

A reaction involving ring closure by still another
route was reported by Piper and Stevens (73). In their syn-
thetic scheme, substituted anthranilic acids (xxix) were

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cyclized to 1-acetyl-chloro-2-methylindoxyl acetates (xxx).

(xxix)   (xxx)

(xxxi)   (xxxii)
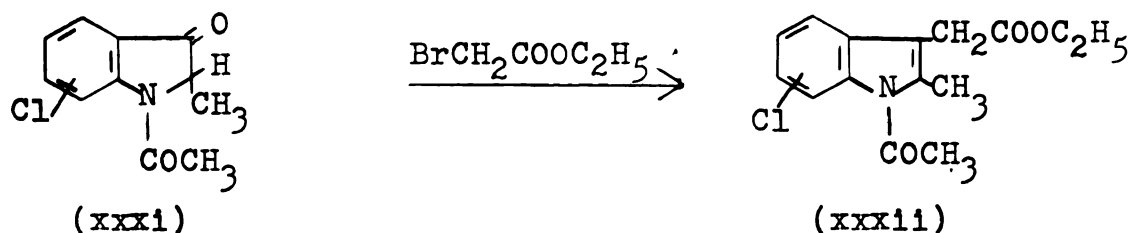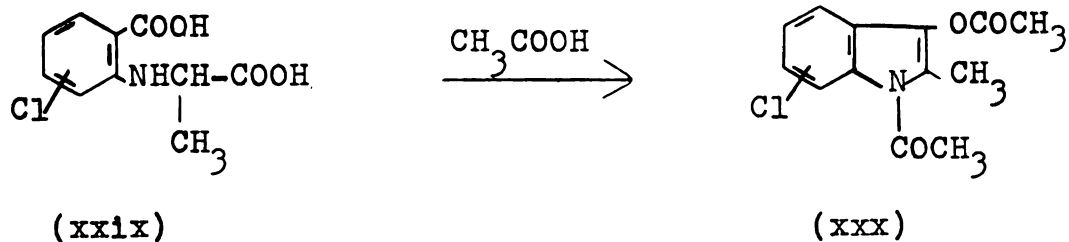
After removal of the 3-acetyl group with sodium sulfite in dioxane and water, the Reformatsky product (xxxii) was obtained by the action of ethyl bromoacetate.

Several observations are pertinent to the considerations of a synthetic sequence involving 1-substituted indoles. One-alkyl indoles will react well with oxalyl chloride to yield the 3-substituted compound, a glyoxyly-chloride (xxxiii) (28).

(xxxiii)   (xxxiv)

(xxxv)

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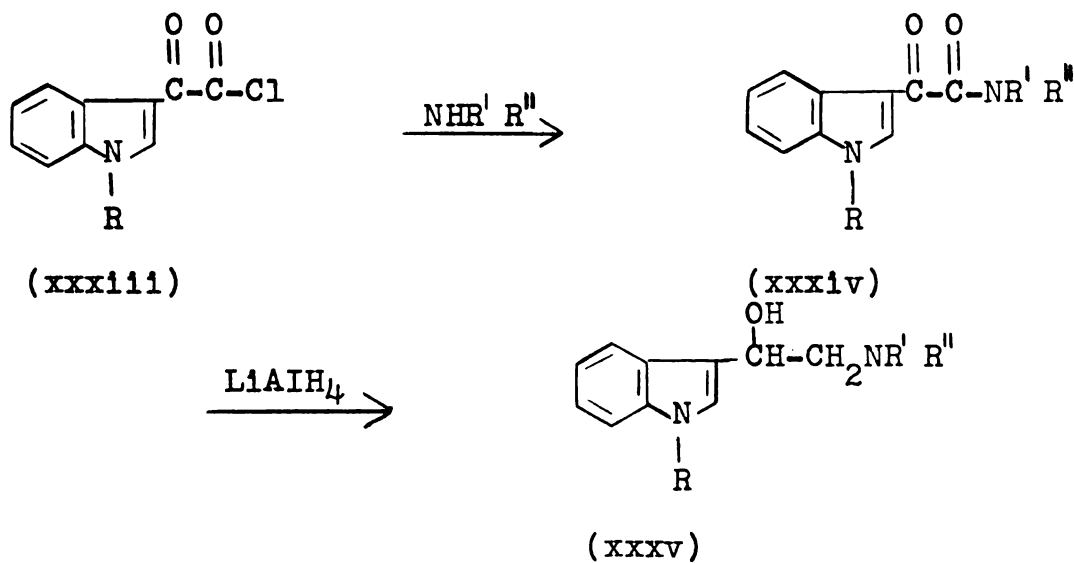
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After amidation, however, the glyoxylylamide will not undergo
the usual reduction to a saturated side-chain which is typical
of indoles with a free 1-position, but yields instead the
alcohol (xxxv) (31). The importance of this synthetic tech-
nique lies in its usefulness for preparing tryptamine analogs.

Another anomalous reaction has been observed with
1-alkyl indoles. In the Mannich reaction of indole with
formaldehyde and dimethylamine, gramine (xxxvii, R = H) is
produced in excellent yield (92).

$$\text{(xxxvi)} \quad \text{R (R = H; alkyl)} \qquad + \quad HCHO + NH(CH_3)_2 \longrightarrow \qquad CH_2N(CH_3)_2 \quad \text{(xxxvii)}$$
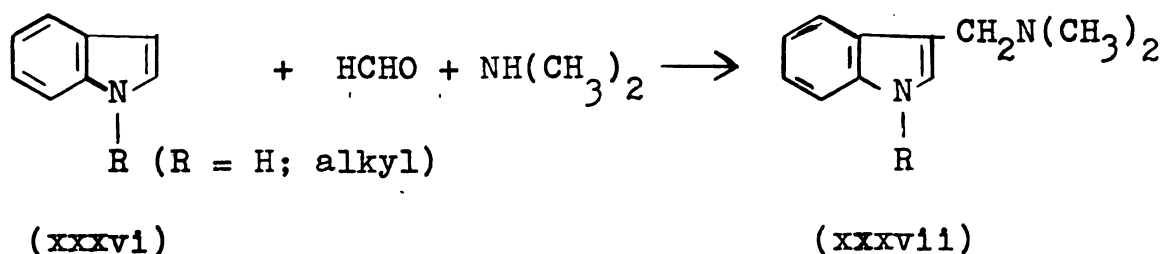
The dimethylamine moiety of gramine may be replaced
with cyanide by direct reaction with sodium cyanide (92) to
yield a mixture of indole-3-acetic acid and indole-3-
acetonitrile. However, when 1-methyl gramine (xxxvii,
R = CH_3) is reacted under similar conditions only starting
material can be isolated (91). Nevertheless, if the
methiodide salt of 1-methyl gramine is used in the reaction
with cyanide, the expected product is obtained. Signifi-
cantly, this product is accompanied by a 2-substituted
nitrile (42, 90).

CHEMICAL STRUCTURE AND PHYSIOLOGICAL ACTIVITY

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CHEMICAL STRUCTURE AND PHYSIOLOGICAL ACTIVITY

Compounds which elicit a certain set of plant responses similar to that produced by indole-3-acetic acid are known as auxins. An acceptable definition for growth regulators was presented in 1951 by a committee of the American Society of Plant Physiologists (112) who defined them as "organic compounds, other than nutrients which in small amounts promote, inhibit, or otherwise modify any physiological process in plants." Auxins as a subclass of the above were defined as "a generic term for compounds characterized by their capacity to induce elongation in shoot cells. They resemble indole-3-acetic acid in physiological action." Unfortunately, cell elongation is not the only observed effect of real auxins. Auxins are in some way involved in differentiation, fruit-set, abscission, and apical dominance, and indole-3-acetic acid as the reference compound can alter all of these processes. The growth regulators are employed in agriculture for such purposes as weed control, fruit-thinning, root induction, and control of flowering.

It is not surprising that synthetic derivatives of indole-3-acetic acid are similar in their action to the parent acid, but it is less obvious that compounds like phenoxyacetic and naphthyleneacetic acids should give a physiological response similar enough to indole-3-acetic acid to be classified as auxins.

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The structural requirements which are necessary for biological activity have been sought for many years. Early attempts to enumerate the essential factors were made by Koepfli, Thimann, and Went in 1938 (47) who listed five structural requirements:

(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 the side-chain at least one carbon removed from the ring

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

As new compounds were discovered and assayed, it became apparent that these requirements were too confining. The activity exhibited by the halogenated benzoic acids removed the requirement for a side-chain. Only two general requirements were considered necessary by Veldstra and Booij (116), who studied the relationship of chemical structure to biological activity in many compounds and proposed the following requirements for activity:

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

(b) a carboxyl (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.

Numerous exceptions to these condensed rules, however, have been observed. Among these are the observations by

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Veldstra (114, 117) that primary nitro or sulfonic acid groups
will substitute for the carboxyl as will phosphonous and phos-
phonic acid derivatives. The tetrazole analog of indole-3-
acetic acid, 5-(3'-indole-methyl)-tetrazole, also exhibits
slight auxin activity. Because tetrazoles of this type have
approximately the same pK_a values as do aryl carboxylic acids
(30), this could account for a similar character. In each of
the cases mentioned, the group that replaced the carboxyl had
some degree of anionic charge indicating the important aspect
of this structure. Significantly, anionic groups other than
the carboxyl moeity impart a much lower degree of activity
than do the carboxylic acids.

Even certain compounds without a ring structure show
auxin activity. Thus, 5-(carboxymethyl)-dimethyldithio-
carbamic acid was observed to cause growth of cucumber,
tomato, and pea plants (113). Compounds with this unique
structure may assume a planar configuration and probably do
so in the resonance hybrid:

$$
\underset{(A)}{\begin{array}{c} CH_3 \\ \diagdown \ddot{N}-\overset{\overset{\displaystyle S}{\|}}{C} \\ CH_3 \diagup \qquad \diagdown S-CH_2COOH \end{array}}
\quad\longleftrightarrow\quad
\underset{(B)}{\begin{array}{c} CH_3 \\ \diagdown \overset{+}{N}=\overset{\overset{\displaystyle \ddot{S}\,-}{|}}{C} \\ CH_3 \diagup \qquad \diagdown S-CH_2COOH \end{array}}
$$
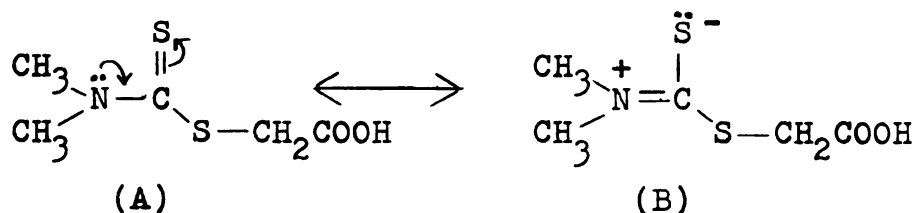
According to Pitzer (74) only the first row elements
produce strong multiple bonds. The formation of such bonds
with heavier elements is hampered since they lack the capacity
to bond in this way due to the "inner shell repulsion" of the
completed inner octets. Therefore, the resonance contributor

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(B), with its trigonally hybridized sp^2 nitrogen atom, would be more highly favored than the non-planar sp^3 configuration. In addition to possessing the planar structure thought to be necessary for an auxin response, the contributor (B) would have the double bond and complement of pi electrons required for high surface activity as specified by Veldstra. Apparently the demand for an unsaturated ring must be modified as a requirement for a planar unsaturated structure.

In 1938, Thimann and Bonner (106) showed that the activity of indole alkanoic acids alternated with side-chain extension. Even numbered carbon chains always showed a higher activity than the odd numbered counterparts. The even numbered chains are degraded to indole-3-acetic acid by β-oxidation while the odd numbered chains yield the inactive indole-3-carboxylic acid.

At the time of the investigation, however, the process of β-oxidation in fatty acid metabolism remained to be elucidated. Wightman (124) investigated the β-oxidation of indole-3-alkanoic acids with chains up to seven carbons in length. He was able to demonstrate that acids with even numbered chains were degraded to indole-3-acetic acid. The longer even members also gave appreciable quantities of indole-3-butyric acid, an indication of β-oxidation. Odd numbered acids gave only indole-3-propanoic acid in measureable quantities and none of the expected indole-3-carboxylic acid could be detected.

Thimann and his coworkers (76, 105) proposed that a critical property of auxins is the spacial relationship

between the anionic charge on the carboxy group and a par-
tially positive charge located 5.5 angstroms from the car-
bonyl group. In the indole auxins the cationic center is
the nitrogen of the pyrrole ring. The position of the
charge centers and their orientation is shown in Figure A
(105). Indole-3-acetic acid is compared with the most
active auxins of three other classes.
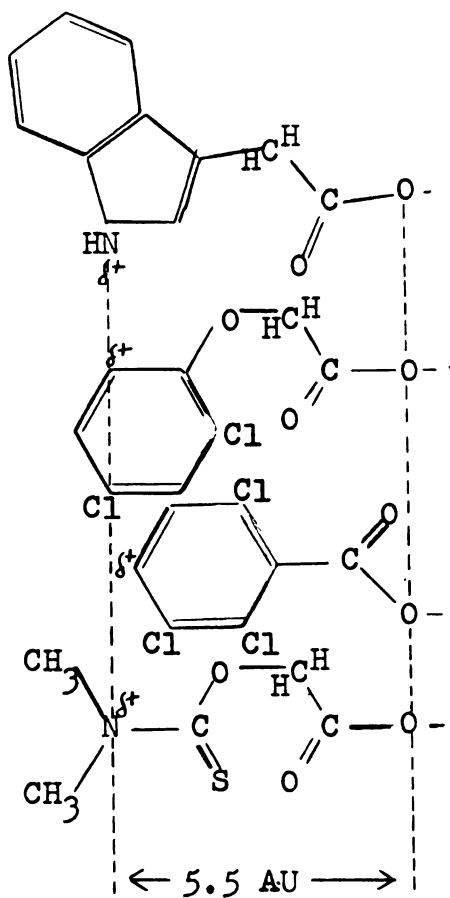
Figure A

More recently, Porter and Thimann (76) have postulated the
importance of the extent of partially positive charge on the
degree of auxin activity. Indole-3-acetic acid substituted

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in the 2-position with either chlorine or bromine has an auxin activity of 350% and 160% respectively, relative to the parent compound. Significantly, similar halogenation of lysergic acid diethylamide greatly increases its activity as a serotonin antagonist in animal systems (111).

Other work seems to support Thimann's hypothesis. Sell et al. (86) and Ritzert et al. (81) have assayed 1-benzoyl derivatives of indole-3-acetic acid and found them to be quite active in tomato fruit-set. The latter workers also noted considerable Avena straight growth activity. 1-Benzoyl derivatives would be expected to delocalize the lone pair of electrons on the nitrogen and enhance the partially positive charge. In opposition to this enhancement would be the steric requirement of the 1-substituent. With the data at hand, one cannot be sure that the derivatives acted per se and were not degraded to indole-3-acetic acid.

Veldstra (115) showed that the nitrogen atom of indole-3-acetic acid was not essential for activity. The carbocyclic analog of indole-3-acetic acid, indene-3-acetic acid, as well as the oxygen heterocyclic analog, coumaran-3-acetic acid, show some activity in the Avena straight growth assay. Lower activity was seen in Avena curvature assays, an indication that the synthetic material was not well transported. Substitution of the nitrogen atom with a sulfur atom lowers the activity considerably but does not destroy it (45). Hellman et al. (29) found that insertion

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of an additional nitrogen in the 2 position did not alter activity in <u>Avena</u> straight growth, tomato fruit-set, or cucumber root inhibition assays.

Substituents on the carbon atoms of the rings can cause drastic alteration in activity. The high activity of the 2-halo-derivatives of indole-3-acetic acid has already been mentioned. Chlorination on any of the carbon atoms of indole-3-acetic acid enhances the activity of the molecule (75). Methyl groups, on the other hand, generally lower activity when substituted on the rings. This is particularly true of a methyl group on positions 1, 2, or 7,--positions closely associated with the ring nitrogen atom. With the exception of direct methyl substitution on the nitrogen, it seems unlikely that the electronic effect of this group is of much importance. Insofar as electronic properties are concerned, the methyl group is one of the least powerful donating groups usually encountered (35).

Steric blockage is then the most likely explanation for reduction in activity. Even though the nitrogen is not an absolute necessity and in fact does not exist in some of the most potent auxins, it seems to be implicated as an important center in indole-3-acetic acid.

Polar substituents attached to the auxin molecule almost always decimate the activity. Thimann (107) has shown that two carboxyls on an auxin always destroy activity in the pea test. Among the indoles, indolemethylenemalonic acid showed no activity. Invariably introduction of an

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hydroxyl group drastically lowers activity. Bearing on this
is the demonstrated low activity of the four indole auxins:
5-hydroxy-indole-3-acetic acid, 7-hydroxy-indole-3-acetic
acid, indole-3-lactic acid and indole-3-glycolic acid. A
related compound, indole-3-glyoxylic acid containing an
α-carboxyl group, also showed very low _Avena_ activity.

Perhaps the anionic nature of the substituents causes
them to compete for an attachment site with the side-chain
carboxyl group. The low activity of the hydrophilic
hydroxyl group may be explained by the hypothesis that mem-
branes are somewhat lipoidal and prone to reject hydro-
philic entities. Certainly of major importance in auxin
action are the phenomena of transport and translocation
involving lipid solubility and partitioning, which were
pointed out by Hansch _et al_. (26, 27) in their rather
exhaustive work on phenoxyacetic acids. Hydrophilic indole-
acetylglucose was proposed by Zenk as a detoxification form
of indole-3-acetic acid. Hydrolysis of this conjugate to
yield indole-3-acetic acid may explain its activity.

When Smith and Wain (88) investigated the effect of
various substituents on the side-chain, they proposed that
three factors are important in binding the active compounds
to an active site: (a) an unsaturated ring, (b) a polar
side group, and (c) at least one hydrogen atom on the carbon
in the side-chain. Veldstra (117) found that α-methylene
and α-isopropylidene phenylacetic acids, however, were more
active in the split pea test than was the parent acid.

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Because the hydrogenation product of the latter compound, α-isopropylphenyl acetic acid is inactive, obviously activity cannot be attributed to hydrogenation. This finding would tend to negate the importance of an α-hydrogen atom. In addition, α-dimethylindole-3-acetic acid has been reported to be active in high concentrations (103) even though it is devoid of an α-hydrogen.

Although Fredga and Aberg (19) reviewed the effects of sterioisomerism in auxins, they indicated that very little had been done with indoles. Kögl, the first researcher to study the optically active forms of α-methyl-indole-3-acetic acid, found considerable difference (30 times) between the D(+) and L(-) antipods with the D form more active in the oat coleoptile curvature test (48). However, the oat cylinder test gave identical results with both isomers (100). The general trend is the same in non-indole auxins in that the D form is more active (107). These findings lend support to the theory proposed by Wain (88) concerning three point attachment to the auxin active site.

The contradictory results from the investigations concerning the importance of an α-hydrogen indicate the tentative nature of the present knowledge. In view of the most recent findings, however, Thimann's hypothesis concerning the requirement for a positive center associated with an electron rich planar structure and separated by a given distance from a negative center seems at this time to be the most limiting demand which can be placed on the structure of an auxin.

BIOLOGICAL ASSAY

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BIOLOGICAL ASSAY

A variety of biological assays have been investigated. These assays were designed to examine a particular phenomenon or to minimize side effects caused by certain aspects of plant structure. Thus, the Avena curvature assay (122) was designed to investigate basipetal transport of an applied auxin down one side of an Avena coleoptile and depends upon cell elongation for the resultant curvature. This and other curvature assays such as the split pea assay (116) have been widely used even though the response is more complex than simple cell elongation.

In this work, five assays were used. The Avena straight growth assay (70) is widely employed for testing auxins. This assay has the advantage that penetration and transport effects are held to a minimum. The increase in length of coleoptiles floated on a test solution as compared to those floated on buffer is a measure of auxin activity.

Root inhibition by test compounds also serves as an index of activity. Buckwheat root inhibition was shown by Vitou and Wain (119) to be a reliable and valuable assay for the auxin response. This assay depends upon the inhibition of root growth caused by supraoptimal concentrations of auxin.

Wittwer and Tolbert (125) showed that gibberellins

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and auxins are involved in tomato fruit-set. Active members of either series will cause parthenocarpic growth. Lanolin solutions of the test compounds in question were applied to the ovaries of emasculated flowers and the ovary diameter was measured a few days later.

Bean petiole abscission is a long term assay which is a measure of the ability of the test compound to replace the factor in the intact leaf which maintains a healthy petiole. Lanolin solutions were applied to the severed petiole. The degree of activity was judged by the length of time necessary for abscission to occurr.

The cucumber curvature test (83) serves as a measure of the secondary properties, absorption and translocation in an intact plant. One cotyledon of a cucumber seedling was treated with a lanolin solution of the test substance. A positive response was noted when the plant stem curved away from the treated cotyledon.

Complete details of each assay are presented in the appropriate section.

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EXPERIMENTAL

Synthesis of the Compounds

Reaction of indoline with an alkyl iodide in the presence of anhydrous, powdered sodium carbonate (87) was used to prepare intermediates in the synthesis of several 1-alkyl indoleacetic acids. In this way a 1-alkyl indoline could be prepared which was purified by the action of benzenesulfonyl chloride in base on the unreacted indoline, followed by extraction, and vacuum distillation.

Aromatization of the substituted indoline to a substituted indole was easily accomplished by reacting it with dichlorodicyanobenzoquinone (DDQ). The reaction mixture which employed dichlorodicyanobenzoquinone was found to be much more easily purified and afforded better yields than when chloranil was used. Distillation _in vacuo_ gave the purified indole derivative.

Attachment of the side-chain to yield the 1-substituted ethyl ester of indole-3-acetic acid was accomplished by reaction with ethyl diazoacetate (69, 16). Vacuum distillation always gave a fore-run of unreacted N-substituted indole as well as some lower boiling material. Saponification in alcoholic aqueous sodium hydroxide yielded the appropriate acids in 25-85% yields. Recrystallization from benzene-hexane improved the color in most cases.

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Benzylation of the indolic nitrogen was effected using the method reported in a Merck patent (64) for similar compounds. Using this procedure, a dimethylformamide (DMF) solution of the N-sodium salt of ethyl indole-3-acetate (EIA) was prepared at room temperature by adding a dimethylformamide solution of ethylindole-3-acetate to a stirred slurry of sodium hydride in dimethylformamide. The salt solution was cooled to the temperature of either an ice water bath or a dry ice-acetone bath and a dimethylformamide solution of the p-substituted benzyl halide was added slowly with continual stirring. Careful fractional crystallization and recrystallization gave the desired N-substituted products. However, preparative layer chromatography was found most effective for isolation of pure material. A principle by-product was isolated and shown to be the corresponding p-substituted benzyl ester of indole-3-acetic acid. Ethyl 1-p-nitrobenzyl-indole-3-acetate could not be prepared by this method, and consequently another synthetic route analogous to that used for the preparation of 1-alkyl derivatives was found suitable even though workup was tedious and the yield was minimal. The p-chlorobenzyl derivative was also synthesized by this method and was used to verify the structure of the chloro compound previously described.

Glucose was attached to the 1-position of indole-3-acetic acid (IAA) by preparing 1-(β-D-glucopyranosyl) indole using the synthetic sequence of Suvorov and Preobrazhenskaya (97) except that dichlorodicyanobenzoquinone was employed

instead of chloranil. Ethyl diazoacetate proved satisfactory
for attachment of the ethyl acetate side-chain, but purifica-
tion had to be carried out with column chromatography followed
by crystallization. The acetate blocking groups were removed
with methanolic barium methoxide (34) and the resulting
methyl 1-glucosyl-indole-3-acetate crystallized from methanol-
water. Saponification of the methyl ester with barium hydrox-
ide gave an amorphous precipitate which could be separated
from the starting material with preparative layer chromato-
graphy (PLC) but could not be crystallized.

1-Alkyl Indolines

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I   II   III
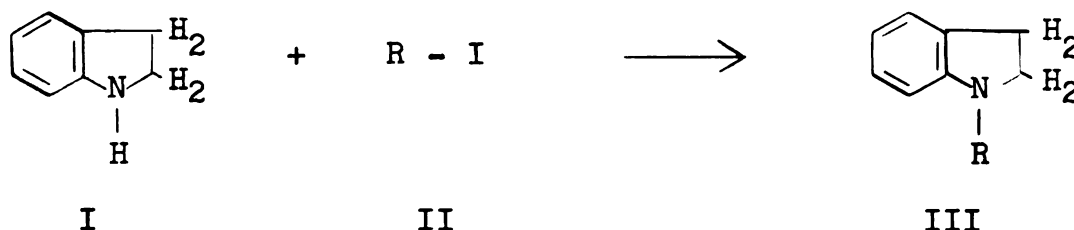
1-Methylindoline (III, R = methyl) (87)

Twenty-three and eight tenths grams (0.2 mole) of
indoline was mixed under stirring with 21.8 g of anhydrous,
powdered sodium carbonate and then approximately 100 ml of
dimethylformamide was added. While cooling to 0°, the slurry
was stirred with a magnetic stirrer. Twenty-five grams
(0.2 mole) of methyl iodide was added and stirring was con-
tinued overnight. The mixture was heated to 100° for 6 hours
and cooled. Then water was added and the organic material
was extracted with chloroform. The chloroform layer was
washed twice with water, dryed with anhydrous calcium chloride,

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filtered, and evaporated to an oil on a Rinco rotary flash evaporator. The impure oil containing unchanged indoline was shaken in a 5% sodium hydroxide suspension with excess benzenesulfonyl chloride until a negative test for the halide was attained. The desired substituted indoline was removed by extraction with ethyl ether from the reaction mixture. The ether solution was extracted with 1 $\underline{N}$ hydrochloric acid several times, and the acid extract neutralized with 5% sodium hydroxide. (The solution clouded noticeably and turned from violet-brown to yellow at the end point.) Re-extracting of the product from the alkaline solution with ether, washing twice with water, drying with anhydrous calcium chloride, filtering, and evaporating gave an oil that distilled at 0.08 mm Hg and $40-44°$. Purity was indicated by lack of the characteristic N-H stretching absorbance (2.9 μ) in the infrared spectrum. The yield was 11.1 g (44%). Ultraviolet absorption showed a bathochromic shift from the parent compound of 5-8 mμ with peaks at 249 mμ and 297 mμ, the former being more prominent. The refractive index was η_D^{25} 1.5670.

1-Ethylindoline (III, R = ethyl) (87)

Ethyl iodide (23.9 g, 0.15 mole) was dropped into an ice-cold, stirred slurry of 14.8 g (0.14 mole) anhydrous, powdered sodium carbonate and 16.6 g (0.14 mole) of indoline. The reaction mixture was stirred for 3 hours and permitted to warm to room temperature. After the mixture was heated for 6 hours on a boiling water bath, it was cooled. Then

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water was added, and the product was extracted twice with
ethyl ether.

The ether layer was first washed twice with water and
then evaporated to an oil which was reacted in 5% sodium
hydroxide with excess benzenesulfonyl chloride until its
odor disappeared. This reaction mixture was extracted twice
with ether. The ether was washed twice with water and
extracted with 1 $\underline{N}$ hydrochloric acid to remove the product.
After the solution was neutralized with 5% sodium hydroxide,
re-extracted with ether, and dryed with anhydrous calcium
chloride, the solvent was evaporated. Then the product was
distilled at 108-110° and 13 mm Hg. Skeinkman and Kost (87)
report 99-102° at 8 mm Hg and the refractive index η_D^{25}
1.5603. Ten grams of product lacking N-H absorption in the
infrared was isolated in a yield of 41%. Physical constants
were η_D^{25} 1.5580 and $\lambda_{max\ (m\mu)}^{ethanol\ (95\%)}$ 252 and 300.

1-n-Propylindole (III, R = n-propyl)

Eleven and nine-tenths grams (0.1 mole) of indoline
(11.9%) was stirred with 10.6 g (0.1 mole) of anhydrous
sodium carbonate at 0° while 17 g (0.1 mole) of $\underline{n}$-propyl
iodide was added dropwise. The slurry was stirred overnight,
then heated to the temperature of a boiling water bath for
6 hours. After addition of water, extraction with ethyl
ether, and evaporation of the solvent, the reaction mixture
was reacted with excess benzenesulfonyl chloride in 5%
sodium hydroxide until the odor of the halide disappeared.
This mixture was extracted with ether and then the ether was

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washed twice with water. This product was extracted from the ether with 1 $\underline{N}$ hydrochloric acid. Neutralization of the acid solution, re-extraction of the product with ether, dehydration of the solvent over calcium chloride, and evaporation afforded an oil which distilled at $50-54^\circ$ at 0.1 mm Hg. The yield of product devoid of N-H stretch in the infrared was 47%. Dimethylformamide could be used as a solvent with little change in yield. Ultraviolet absorption showed $\lambda_{max \ (m\mu)}^{ethanol \ (95\%)}$ 254 and 302. The refractive index was η_D^{25} 1.5478.

1-$\underline{iso}$-Propylindoline (III, R = $\underline{iso}$-propyl) (87)

Thirteen and eight-tenths grams (0.2 mole) of indoline was stirred together with 21.2 g (0.2 mole) of anhydrous, powdered sodium carbonate at 0° while 24.6 g (0.2 mole) $\underline{iso}$-propyl bromide was added drop by drop. The mixture was stirred for 2 days and was finally heated to 100° for 6 hours. Water was added and the mixture was extracted with ethyl ether. After washing the ether twice with water and stripping the solvent, the resulting oil was reacted with excess benzenesulfonyl chloride in 5% sodium hydroxide until the reaction mixture was void of the chloride odor. This mixture was extracted twice with ether. The ether was washed twice with water and then extracted twice with 1 $\underline{N}$ hydrochloric acid. After the acid was neutralized with sodium hydroxide (litmus paper), the product was re-extracted with ether, and then washed 2 times with water, dried over anhydrous calcium chloride, and evaporated to yield an oil.

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Distillation at 46-48° and 0.1 mm Hg gave the product which showed no N-H stretch in the infrared spectrum. The yield was 36% with η_D^{25} 1.5501 and $\lambda_{max\ (m\mu)}^{ethanol\ (95\%)}$ 254 and 302. Sheinkman and Kost (87) give the boiling range as 112-115° at 10 mm Hg and the refractive index as η_D^{25} 1.5585.

1-n-Butylindoline (III, R = n-butyl) (87)

While 15 grams (0.136 mole) of indoline and 13.4 g (0.136 mole) of anhydrous, powdered sodium carbonate were being stirred vigorously at 0°, 26.0 g (0.136 mole) of n-butyl iodide was added drop by drop. After 3 hours at 0°, the mixture was permitted to warm to room temperature and the solution was heated for 6 hours at 100° in an oil bath. Water and ethyl ether were added. The ether phase was extracted twice with water, and evaporated to dryness. The resulting oil was treated with benzenesulfonyl chloride in 4% sodium hydroxide solution until no benzenesulfonyl chloride odor remained. This mixture was extracted twice with ether which was in turn extracted 2 times with water. One normal hydrochloric acid was used to remove the product from the ether layer. After the acid extract was neutralized with 4% sodium hydroxide (litmus paper), ether was used to re-extract the product. The washed and dryed ether solution (anhydrous calcium chloride) was evaporated to dryness and the residual oil distilled in vacuo at 130-131° and 13 mm Hg. Sheinkman and Kost (87) report the boiling range of 128-132° at 12 mm Hg. Their value for the refractive index was η_D^{25} 1.5429. No N-H band was seen in the

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infrared region. Ultraviolet absorbancy was $\lambda_{max}^{\text{ethanol (95\%)}}$ (mμ) 254 and 302. Yield was 45% (10 g) with η_D^{25} 1.5407. Use of dimethylformamide as solvent gave 61%.

Analysis: Calcd. for $C_{12}H_{17}N$: N, 7.99%; Found: N, 7.79%.

1-iso-Butylindoline (III, R = iso-butyl)

Equimolar quantities (0.144 mole) of indoline (17.2 g) and anhydrous, powdered sodium carbonate (16.3 g) were stirred together at 0° while (0.144 mole, 26.5 g) of iso-butyl iodide was added slowly. After the mixture was stirred approximately 4 hours, it was heated to 100° for 6 hours, cooled, and extracted with chloroform from the water phase. Two washings of the chloroform extract with water were followed by evaporation of the chloroform and reaction of the residue with excess benzenesulfonyl cnloride in 5% sodium hydroxide until the odor of the reagent disappeared. Extraction of the basic solution with ethyl ether and washing of the extract was followed by re-extraction of the product from the ether with 1 $\underline{N}$ hydrochloric acid. The acid solution was neutralized with 5% sodium hydroxide and extracted with ether. The ether was washed twice with water and dryed with anhydrous calcium chloride. Evaporation of the ether gave an oil which distilled at 58-60° at 0.1 mm Hg for a yield of 9.4 g (37%) and with an η_D^{25} 1.5379. The infrared showed no N-H band. The compound showed ultra-violet absorption $\lambda_{max\ (m\mu)}^{\text{ethanol (95\%)}}$ 256 and 305.

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Analysis: Calcd. for $C_{12}H_{17}N$: N, 7.99%; Found: N, 7.93%.

1-sec-Butylindoline (III, R = sec-butyl)

Twenty-four and one-half grams (0.205 mole) of indoline and 22 g (0.207 mole) of anhydrous, powdered sodium carbonate were stirred together at $0°$ while (38.4 g, 0.207 mole) sec-butyl iodide was added carefully. After the mixture was stirred approximately 4 hours, it was heated at $100°$ for 6 hours. Upon cooling, water was added and the mixture was extracted twice with chloroform. The chloroform was extracted twice with water and evaporated to an oil which was reacted with benzenesulfonyl chloride in 5% sodium hydroxide. When the odor of benzenesulfonyl chloride had disappeared, the reaction mixture was extracted twice with ethyl ether and the ether in turn with 1 $\underline{N}$ hydrochloric acid. Neutralization of the acid extract with 5% sodium hydroxide and re-extraction with ether yielded a solution of the product which could be isolated by drying and evaporating the solvent and then distilling the residue at $64\text{-}66°$ at 0.2 mm Hg. This product had no N-H stretch band in its infrared spectrum. Yield was 12.5 g (35%) with $\lambda_{max\ (m\mu)}^{ethanol\ (95\%)}$ 257 and 308. Its refractive index was η_D^{25} 1.5379.

Analysis: Calcd. for $C_{12}H_{17}N$: N, 7.99%; Found: N, 7.88%.

1-tert-Butylindoline (III, R = tert-butyl)

One hundred grams (0.54 mole) of tert-butyl iodide

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was added dropwise to a cooled (0°), stirred slurry of 32.5 g
(0.5 mole) of indoline and 53 g (0.5 mole) of finely divided
anhydrous sodium carbonate. The solution was warmed to room
temperature and the reaction was brought to completion by
heating approximately 6 hours on a hot water bath ($60-80^{\circ}$)
until a solid mass formed. Water was added and the mixture
was extracted twice with ethyl ether. After several wash-
ings with water, the ether was evaporated and the residue
was reacted with 1 l of 5% sodium hydroxide and 60 ml of
benzenesulfonyl chloride. When the reaction was complete,
the mixture was extracted twice with ether and the ether
was extensively washed and decanted to remove a solid
material. Two extractions of the ether with 5% hydrochloric
acid and neutralization with 5% sodium hydroxide followed by
re-extraction of the aqueous phase with ether and evapora-
tion gave an oil which boiled at $125-127^{\circ}$ and 13 mm Hg.
Yield was 5.5 g (11.5%) of product having only a little N-H
stretch in its infrared spectrum. The analytical sample was
purified on preparative layer chromatography (2 mm silica
gel G in 18% butanone-hexane). Physical data were $\lambda_{max (m\mu)}^{ethanol}$ (95%)
256 and 299; η_D^{25} 1.5431.

Analysis: Calcd. for $C_{12}H_{17}N$: N, 7.99%; Found:
N, 7.97%.

1-n-Pentylindoline (III, R = n-pentyl)

Thirty and six-tenths grams of 1-iodopentane (0.154
mole) was allowed to drip into a stirred suspension of 13 g

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(0.154 mole) of indoline and 15.7 g (0.154 mole) anhydrous, powdered sodium carbonate at 0°. After being warmed to room temperature, the reaction mixture was heated to 100° for 6 hours, cooled, diluted with water, and extracted with chloroform. The organic liquid phase was washed twice with water and evaporated to an oil which was reacted in 5% sodium hydroxide with excess benzenesulfonyl chloride. Then the product was extracted with ethyl ether, the ether was washed twice with water, and the solvent was dryed with anhydrous magnesium sulfate. Subsequent evaporation to dryness followed by distillation at 75-81° and 0.2 mm Hg gave a 13 g yield (45%). Observed constants were η_D^{25} 1.5318 and $\lambda_{max\ (m\mu)}^{ethanol\ (95\%)}$ 254 and 302. Infrared spectral analysis showed no N-H stretch.

Analysis: Calcd. for $C_{13}H_{19}N$: N, 7.40%; Found: N, 7.29%.

1-n-Decylindoline (III, R = n-decyl)

Twenty-three and eight-tenths grams (0.2 mole) of indoline was stirred together with 21.1 g (0.2 mole) of dry, powdered, sodium carbonate at 0° while 53.6 g (0.2 mole) 1-iododecane was added dropwise. The resulting mixture was heated to 100° for 6 hours, cooled, and extracted with chloroform. Water soluble material was removed from the chloroform extract by two washings with water. This extract was dryed with anhydrous magnesium sulfate and evaporated to an oil which was distilled at 132° and 0.3 mm Hg. The yield

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of product was 48.3 g (80%) with η_D^{25} 1.5164. No N-H band

was seen in the infrared spectrum. The ultraviolet spectra

was $\lambda_{max\ (m\mu)}^{ethanol\ (95\%)}$ 256 and 305.

Analysis: Calcd. for $C_{18}H_{29}N$: N, 5.40%; Found:

N, 5.51%.

1-n-Octadecylindoline (III, R = n-octadecyl)

Seven and eighty-five hundredths grams (0.066 mole)

indoline was vigorously stirred with an equimolar quantity

of anhydrous, powdered sodium carbonate (7 g) and n-octa-

decyl iodide (25 g) at room temperature for $9\frac{1}{2}$ hours and

heated to 100° for 6 hours. Water was added and the reac-

tion mixture was extracted twice with chloroform. The

chloroform solution was washed twice with water. Drying

was accomplished with anhydrous sodium sulfate and the

solvent removed in vacuo. Unreacted indoline and some

solid material was recovered in the lower boiling fractions

and necessitated dismantling the distillation apparatus for

cleaning before the final distillation of the product at

209° and 0.25 mm Hg. The product crystallized in the

receiver, mp 33.5-34.5°. Yield was 63% (1.52 g) with

$\lambda_{max\ (m\mu)}^{ethanol\ (95\%)}$ 254 and 300. The refractive index was η_D^{35}

1.4954. No N-H stretch was noted in the infrared spectrum.

Analysis: Calcd. for $C_{26}H_{45}N$: N, 3.77%; Found:

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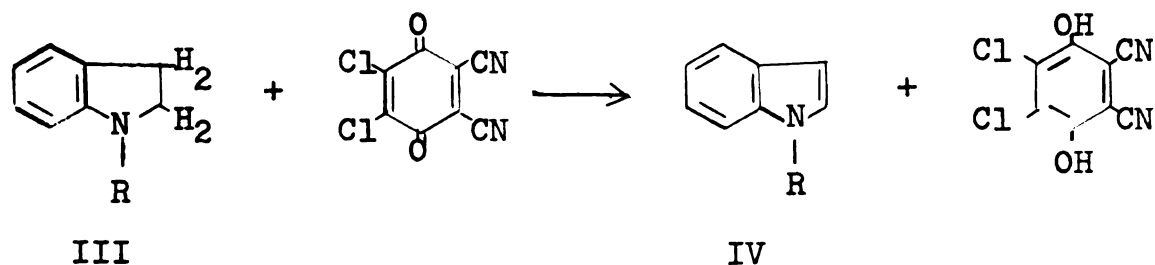
1-Methylindole (IV, R = methyl)

Ten and six-tenths grams (0.08 mole) of methyl indoline was dissolved in approximately 250 ml of dry xylene and an equimolar quantity of dichlorodicyanobenzoquinone was added. The addition of dichlorodicyanobenzoquinone caused considerable heating. When solution was complete, the mixture was refluxed for 6 hours, cooled, and filtered. The solvent was removed under reduced pressure. The residue was distilled at 58-65° under 0.6 mm Hg and yielded 6.6 g (63%). The refractive index was η_D^{25} 1.5945. The infrared spectrum was devoid of N-H stretching absorbance. Ultraviolet absorbance showed $\lambda_{max\ (m\mu)}^{ethanol\ (95\%)}$ 274, 282 and 294. Noland et al. (71) report boiling point 72° at 0.85 mm Hg for the product obtained from the reaction of methyl iodide and sodium amide with indole in liquid ammonia. Gray and Archer (21) report η_D^{25} 1.6038.

1-Ethylindole (IV, R = ethyl)

1-Ethylindoline (8.9 g, 0.060 mole) and 13.7 g (0.060 mole) of dichlorodicyanobenzoquinone were dissolved in approximately 250 ml dry xylene and brought to reflux. After heating 6 hours, the suspension was cooled, filtered to remove

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the by-product quinone, and evaporated on a flash evaporator. The resulting product distilled at 114-120° and 13 mm Hg to yield 4.3 g (48%) of the product with η_D^{25} 1.5860 and $\lambda_{max\ (m\mu)}^{ethanol}$ (95%) 274, 282 and 294. No infrared absorbance attributable to the N-H group was observed. Gray et al. (22) report a boiling point of 82-85° and a refractive index η_D^{26} 1.5889.

1-n-Propylindole (IV, R = n-propyl)

n-Propylindoline (8.05 g, 0.05 mole) and an equimolar quantity (11.4 g) of dichlorodicyanobenzoquinone were dissolved in approximately 200 ml of dry xylene. After all solid material had dissolved, the solution was refluxed for 6 hours. Upon cooling, the solution was filtered to remove the by-product and evaporated. The resulting oil distilled at 64° and 0.15 mm Hg. No N-H stretch absorbance was seen in the infrared region. The typical bathochromic shift was observed in the ultraviolet spectrum (126) with $\lambda_{max\ (m\mu)}^{ethanol}$ (95%) 276, 282, and 294. The refractive index was η_D^{25} 1.5704 on a product obtained in 45% yield (3.6 g).

1-iso-Propylindole (IV, R = iso-propyl)

A solution of 9.8 g (0.061 mole) of iso-propyl indoline with 14.9 g (0.061 mole) chloranil in approximately 250 ml dry xylene was brought to reflux and heated for 6 hours with stirring. After the solution was cooled and filtered, it was washed once with dilute sodium hydroxide to remove some of the substituted hydroquinone, twice with dilute hydrochloric acid to remove unreacted substituted indoline, and

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finally with water. When the solvent had been removed _in vacuo_, ethyl ether was added and the solution was washed again with dilute sodium hydroxide and with water. Anhydrous magnesium sulfate was used to dry the solution which was then evaporated to an oil and distilled at 69° and 0.08 mm Hg. Yield was 6.1 g (48%) of an oil with η_D^{25} 1.5755. Ultraviolet absorption showed $\lambda_{max\ (m\mu)}^{ethanol\ (95\%)}$ 276, 283, 293. No N-H band was seen in the infrared spectra. Michaelis (66) gave the boiling point as 250°.

1-n-Butylindole (IV, R = n-butyl)

Nine and one-half grams (0.054 mole) of 1-n-butylindoline plus 12.3 g (0.541 mole) of dichlorodicyanobenzoquinone in a solution of approximately 250 ml dry xylene was refluxed 6 hours, cooled, filtered, and evaporated to an oil which was distilled at $139-145^{\circ}$ at 13 mm Hg. The product was obtained in 60% yield (5.8 g) and showed no N-H stretch in the infrared spectrum. The η_D^{25} was 1.5628 with $\lambda_{max\ (m\mu)}^{ethanol\ (95\%)}$ 275, 282, and 294.

1-iso-Butylindole (IV, R = iso-butyl)

A solution of 13.1 g (0.0535 mole) chloranil and 9.25 g (0.0530 mole) 1-iso-butylindoline was refluxed 8 hours. After cooling, filtering, and washing successively several times with dilute sodium hydroxide, dilute hydrochloric acid, and water, respectively, the solution was dryed (magnesium sulfate), filtered with a little Norit A through Hiflow Supercel and finally evaporated. The residual oil distilled

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at 75° and 0.5 mm Hg for a yield of 5.05 g (54%) with η_D^{25} 1.5626. Absorption spectra showed $\lambda_{max\ (m\mu)}^{ethanol\ (95\%)}$ 276, 283 and 294. No N-H stretch was observed in the infrared spectrum.

Analysis: Calcd. for $C_{12}H_{15}N$: N, 8.08%; Found: N, 7.91%.

1-sec-Butylindole (IV, R = sec-butyl)

To 11.7 g (0.067 mole) sec-butylindoline in approximately 250 ml dry xylene was added 15.2 g (0.067 mole) of dichlorodicyanobenzoquinone. When solution was complete, the reaction mixture was refluxed for 6 hours, cooled, and filtered. The filtrate was evaporated to an oil. After distillation at 69° and 0.08 mm Hg, it gave 6.5 g (54%) of a product free of N-H stretch in the infrared. The η_D^{25} was 1.5636 after preparative layer chromatography. Ultraviolet absorption showed $\lambda_{max\ (m\mu)}^{ethanol\ (95\%)}$ 273, 282 and 294.

Analysis: Calcd. for $C_{12}H_{15}N$: N, 8.08%; Found: N, 7.70%.

1-tert-Butylindole (IV, R = tert-butyl)

tert-Butylindoline (1.6 g, 0.0103 mole) and 2.34 g (0.0103 mole) of dichlorodicyanobenzoquinone were dissolved in 25 ml xylene and refluxed 6 hours. After the solution was cooled, it was filtered and evaporated. The product was distilled from the residue to yield 1.3 g (67%). Boiling point was 75° at 0.08 mm Hg. The $\lambda_{max\ (m\mu)}^{ethanol\ (95\%)}$ was 274, 281, 292. The η_D^{25} was 1.5708 after preparative layer chromatography. Only a very small N-H band was noted in the

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Analysis: Calcd. for $C_{12}H_{15}N$: N, 8.08%; Found: N, 7.97%.

1-n-Pentylindole (IV, R = n-pentyl)

A solution of 8.4 g (0.0444 mole) of n-pentylindoline and 10.1 g (0.0446 mole) of dichlorodicyanobenzoquinone in about 200 ml dry xylene was refluxed for 6 hours. After the solution was cooled and filtered to remove the by-product, the solvent was evaporated. The resulting product was distilled at 82-95° and 0.07-0.08 mm Hg. The yield was 53% or 4.3 g with an η_D^{25} 1.5537. Infrared data was consistent with the assigned structure. Ultraviolet absorption was $\lambda_{max\ (m\mu)}^{ethanol\ (95\%)}$ 273, 283, and 294.

Analysis: Calcd. for $C_{13}H_{17}N$: N, 7.48%; Found: N, 7.41%.

1-n-Decylindole (IV, R = n-decyl)

After dissolving 11.45 g (0.0443 mole) of n-decylindoline in 300 ml dry xylene, 10 g (0.0443 mole) of dichlorodicyanobenzoquinone was added. When solution was complete, the reaction mixture was refluxed for 6 hours. The solution was cooled, filtered, and evaporated, yielding an oil which distilled at 136-143° and 0.1 mm Hg. Six g (52%) of product was obtained with an η_D^{25} 1.5264. No N-H band was present in the infrared spectrum. In the ultraviolet, absorbance was $\lambda_{max\ (m\mu)}^{ethanol\ (95\%)}$ 270, 283, and 294.

Analysis: Calcd. for $C_{18}H_{27}N$: N, 5.44%; Found: N, 5.39%.

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A similar procedure was followed using 28 g (0.108 mole) of n-decylindoline and 26.6 g (0.108 mole) chloranil in 500 ml xylene. The xylene filtrate was washed extensively in a vain attempt to remove the very dark colored materials and finally dryed with anhydrous magnesium sulfate. Next it was filtered and evaporated. Distillation of the resulting oil gave 8.25 g (29%) of product having properties similar to that of the material made with dichlorodicyanobenzoquinone.

1-n-Octadecylindole (IV, R = n-octadecyl)

Refluxing a solution made of 15.2 g of (0.0412 mole) n-octadecylindoline and 9.35 g (0.0412 mole) dichlorodicyano-benzoquinone in 200 ml xylene for 6 hours resulted in oxidation of the substituted indoline. Isolation involved filtration of the cooled slurry and evaporation of the filtrate to an oil which distilled at 210-225° and 0.1 mm Hg. During the procedure, some solid by-product had to be removed from the still before distillation could be completed. The oil was filtered to remove a small amount of solid and was redistilled for a net yield of 11 g (59%). The η_D^{25} was 1.5108 with $\lambda_{max\ (m\mu)}^{ethanol\ (95\%)}$ 276, 283, and 294.

Analysis: Calcd. for $C_{26}H_{43}N$: N, 3.79%; Found: N, 3.77%.

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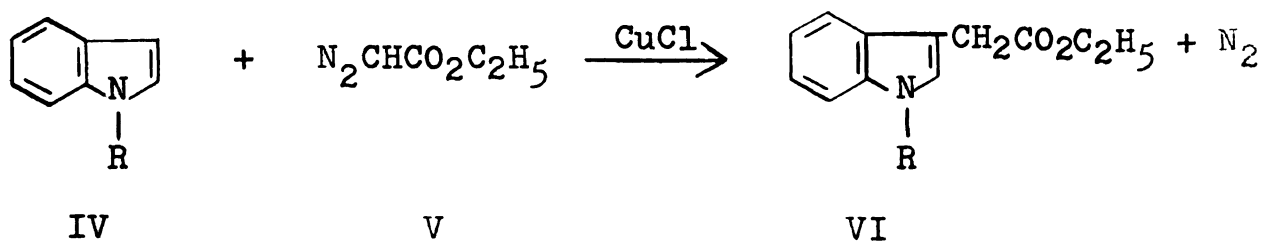
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Ethyl Diazoacetate (V)

A solution of 210 g (1.5 mole) of the hydrochloride salt of ethyl glycine ester and 1.05 g (0.0129 mole) sodium acetate in 220 ml of water was cooled in a large beaker in an ice bath. While the solution was stirring, 157.5 g (2.3 mole) of sodium nitrite in 220 ml water was slowly added, followed by 125 ml of ether. The bath was maintained below 20° while 25 ml of a 10% solution of sulfuric acid was added slowly with continued stirring. In about 30 minutes the reaction was complete and the liquid was then drawn into a separatory funnel with vacuum. The aqueous layer was returned to the reaction beaker. The ether solution was immediately washed with cold 10% sodium carbonate. Twenty-five ml of 10% sulfuric acid was added to the reaction mixture and it was re-extracted with 100 ml of ether. This ether extract was also washed with the cold aqueous sodium carbonate. The entire procedure was then repeated with 75 ml of 10% sulfuric acid solution and 100 ml of ether. The combined washed ether extracts were washed with a saturated sodium chloride solution. Anhydrous sodium sulfate was used for drying and the ether was distilled _in vacuo_. Blowing dry nitrogen through the solution removed the remaining traces of ether. The yield was 144 g (84%) of a light yellow oil which could be stored in the cold in a dark bottle for over 1 year without loss of activity.

Ethyl 1-methylindole-3-acetate (VI, R = methyl)

A solution of 2.1 g (0.016 mole) 1-methylindole in

approximately 15 ml of dry benzene was heated to boiling on a steam bath and a few milligrams of cuprous chloride was added. A reflux condenser was fitted to the flask and a small dropping funnel placed in the top of the condenser. The dropping funnel was charged with an equimolar quantity (1.83 g) of ethyl diazoacetate in approximately 10 ml of dry benzene. Careful addition of the ester solution resulted in steady evolution of nitrogen. When addition was completed, refluxing was continued for 3 to 4 hours, and the resulting brown solution was filtered. The filtrate was evaporated to an oil which was distilled at 125-130° and 0.09 mm Hg. Yield was 1.6 g (52%) and the η_D^{25} 1.5580. Ultraviolet absorption was $\lambda_{max\ (m\mu)}^{ethanol\ (95\%)}$ 277s, 287 and 298s. The product showed no N-H stretch but had intense C=O (5.80 μ) and ROC=O (8.65 μ) bands in the infrared region. King and L'Ecuyer (46) give a boiling point of 165° at 1 mm Hg; Julia and Tchernoff (40) report 155-60° at 1 mm.

Ethyl 1-ethylindole-3-acetate (VI, R = ethyl)

A solution of 2.66 g (0.0183 mole) 1-ethylindole in approximately 15 ml dry benzene was heated to boiling on a steam bath and a few milligrams of cuprous chloride was added. Two and one-fourth grams (0.0197 mole) ethyl diazo-acetate in 10 ml dry benzene was added dropwise through a reflux condenser at such a rate as to allow the evolved nitrogen to escape without undue foaming. When addition was completed, the solution was allowed to continue reflux-ing for another 4 hours. Cooling, filtering, evaporating

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Yield was 1.6 g (38%) with η_D^{25} 1.5492. Absorption spectra included: $\lambda_{max\ (m\mu)}^{ethanol\ (95\%)}$ 277s, 288 and 297s; $\lambda_{max\ (\mu)}^{neat}$ 5.9 (C=O), 8.69 (ROC=O), and no N-H (2.9). Julia and Tchernoff (40) report the boiling point as 165-170° at 1 mm Hg.

Ethyl 1-n-propylindole-3-acetate (VI, R = n-propyl)

Two and thirty-eight hundredths grams (0.15 mole) of 1-n-propylindole dissolved in dry benzene and brought to reflux on a steam bath was reacted in the presence of a catalytic amount of cuprous chloride with 1.71 g (0.015 mole) of ethyl diazoacetate. Thus, the ester in a solution of 10 ml of dry benzene was added dropwise at such a rate that the nitrogen formed was easily evolved through the reflux condenser. Refluxing was continued for 4 hours. Then the solution was cooled, filtered, and evaporated to an oil which was distilled at 130-140° at 0.12 mm Hg. The yield was 1.25 g (34%) of an oil with η_D^{25} 1.5455. Ultraviolet and infrared data were: $\lambda_{max\ (m\mu)}^{ethanol\ (95\%)}$ 178s, 288, and 298s; $\lambda_{max\ (\mu)}^{neat}$ 5.79 (C=O), 8.69 (ROC=O) with no N-H band around 2.9.

Analysis: Calcd. for $C_{15}H_{19}NO_2$: N, 5.71%; Found: N, 5.76%.

Ethyl 1-iso-propylindole-3-acetate (VI, R = iso-propyl)

To a refluxing solution of 4.5 g (0.0282 mole) 1-iso-propylindole in dry benzene (15 ml) was added a catalytic amount of cuprous chloride. Dropwise addition of 3.18 g

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(0.0279 mole) ethyl diazoacetate in 10 ml dry benzene followed by a reflux period of 4 hours resulted in a solution which was filtered, evaporated, and distilled to yield the product. Three and four-tenths grams (44%) was collected after distillation at 128-138° and 0.15 mm Hg. After purification of an analytical sample on preparative layer chromatography, the η_D^{25} was 1.5462 and the absorption spectra showed: $\lambda_{max\ (m\mu)}^{ethanol}$ (95%) 277s, 287, and 298s; $\lambda_{max\ (\mu)}^{neat}$ 5.79 (C=O), 8.64 (ROC=O), with no N-H band near 2.9.

Analysis: Calcd. for $C_{15}H_{19}NO_2$: N, 5.71%; Found: N, 5.67%.

Ethyl 1-n-butylindole-3-acetate (VI, R = n-butyl)

Ethyl diazoacetate (5.6 g, 0.049 mole) in 15 ml dry benzene was added dropwise to a reflux dry benzene solution of 5.7 g (0.033 mole) of 1-n-butylindole containing a few mgs of cuprous chloride. The rate of addition allowed for a slow steady evolution of nitrogen. After addition was completed, the solution was refluxed for 4 more hours. The oil which resulted after the solution was filtered and evaporated distilled at 159-165° and 0.5 mm Hg. A yield of 3.9 g (45%) was obtained with η_D^{25} 1.5384. Ultraviolet absorption was $\lambda_{max\ (m\mu)}^{ethanol}$ (95%) 278s, 287, and 297s; the infrared region showed $\lambda_{max\ (\mu)}^{neat}$ 5.79 (C=O), 8.68 (ROC=O), and no band at 2.9 (N-H).

Analysis: Calcd. for $C_{16}H_{21}NO_2$: N, 5.40%; Found: N, 5.59%.

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Ethyl 1-iso-butylindole-3-acetate (VI, R = iso-butyl)

Slow addition of 1.61 g (0.0141 mole) of ethyl diazo-acetate in 10 ml of dry benzene to a refluxing solution of 2.45 g (0.0141 mole) 1-iso-butylindole in 10 ml dry benzene gave after isolation 1.5 g (41%) of the product. The solution was fully reacted by refluxing for 4 hours, cooling, filtering, and evaporating to a small volume. The product was finally distilled at 130-145° and 0.3 mm Hg to yield a light oil. Physical constants found were: η_D^{25} 1.5390; $\lambda_{max\ (m\mu)}^{ethanol\ (95\%)}$ 277s, 287, and 297s; $\lambda_{max\ (\mu)}^{neat}$ 5.79 (C=O), 8.62 (ROC=O) with no band near 2.9 (N-H).

Analysis: Calcd. for $C_{16}H_{21}NO_2$: N, 5.40%; Found: N, 5.54%.

Ethyl 1-sec-butylindole-3-acetate (VI, R = sec-butyl)

Five and eight-tenths grams (0.0333 mole) of 1-sec-butylindole in 15 ml dry benzene was refluxed on a steam bath and a catalytic amount of cuprous chloride was added. Slow addition of a solution of 3.82 g (0.0333 mole) of ethyl dizaoacetate in benzene was begun and continued at such a rate that nitrogen was smoothly evolved. The solution was heated for 4-6 hours longer, cooled, filtered, and concentrated in vacuo to an oil. The resultant oil distilled at 135-145° and 0.1 mm Hg. The yield was 3.6 g (41%). Absorption spectra showed $\lambda_{max\ (m\mu)}^{ethanol\ (95\%)}$ 277s, 288, and 298s; $\lambda_{max\ (\mu)}^{neat}$ 5.78 (C=O), 8.61 (ROC=O), no band near 2.9 (N-H). The refractive index was η_D^{25} 1.5416.

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Analysis: Calcd. for $C_{16}H_{21}NO_2$: N, 5.40%; Found:
N, 5.78%.

Ethyl 1-tert-butylindole-3-acetate (VI, R = tert-butyl)

A dry benzene solution (10 ml) of 1-tert-butylindole
(2.13 g, 0.0123 mole) was brought to boiling on a steam
bath and a few mgs of dry cuprous chloride were added. To
this mixture was carefully added a solution of 10 ml of
benzene in which was dissolved 2.1 g (0.0185 mole) ethyl
diazoacetate. After addition was completed, refluxing was
continued for 2 hours. The solution was cooled, filtered,
evaporated, and the residue distilled at 127-140° at 0.09
mm Hg. One and six-tenths grams (67%) of the substituted
ester was obtained with an η_D^{25} of 1.5450. Purification of
the analytical sample on preparative layer chromatography
gave a product with η_D^{25} 1.5431. Absorption data include:
$\lambda_{max (m\mu)}^{ethanol (95\%)}$ 277s, 285, and 295s; $\lambda_{max (\mu)}^{neat}$ 5.78 (C=O)
and 8.65 (ROC=O). No band at 2.9 (N-H) was seen.

Analysis: Calcd. for $C_{16}H_{21}NO_2$: N, 5.40%; Found:
N, 5.55%.

Ethyl 1-n-pentylindole-3-acetate (VI, R = n-pentyl)

Dropwise addition of 2.15 g (0.0189 mole) ethyl diazo-
acetate in 10 ml of dry benzene to a refluxing solution of
1-n-pentylindole (4 g, 0.0214 mole) containing a catalytic
amount of dry cuprous chloride was continued at such a rate
as to allow for constant evolution of the nitrogen by-product.
Four hours after all of the ester had been added, the solution

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was cooled and filtered. Removal of the benzene in vacuo

was followed by distillation at 140-160° under 0.09 mm

pressure to yield 2.6 g (44%) of the product. The clear

yellow oil showed absorption as follows: $\lambda_{max\ (m\mu)}^{ethanol\ (95\%)}$

277s, 288, and 298s; $\lambda_{max\ (\mu)}^{neat}$ 5.79 (C=O), 8.70 (ROC=O),

and no N-H band near 2.9. The refractive index was η_D^{25}

1.5326.

Analysis: Calcd. for $C_{17}H_{23}NO_2$: N, 5.12%; Found:

N, 5.21%.

Ethyl 1-n-decylindole-3-acetate (VI, R = n-decyl)

To a refluxing dry benzene (15 ml) solution of 6 g

(0.019 mole) of 1-n-decylindole was added a catalytic

quantity of anhydrous cuprous chloride. Then addition of

2.6 g (0.023 mole) of ethyl diazoacetate in 10 ml benzene

was begun. After addition of the ester, the solution was

refluxed for an additional 3 hours. Filtration and evapor-

ation of the solution was followed by distillation of the

residue at 166-188° and 0.1 mm Hg for a yield of 3.25 g

(50%). The refractive index was η_D^{25} 1.5164 after prepara-

tive layer chromatography purification. Absorption data

include: $\lambda_{max\ (m\mu)}^{ethanol\ (95\%)}$ 277s, 287, and 297s; $\lambda_{max\ (\mu)}^{neat}$

5.79 (C=O), 8.70 (ROC=O), and no N-H near 2.9.

Analysis: Calcd. for $C_{22}H_{33}NO_2$: N, 4.08%; Found:

N, 4.19%.

Ethyl 1-n-octadecylindole-3-acetate (VI, R = n-octadecyl)

1-n-Octadecylindole (8.5 g, 0.023 mole) dissolved in

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approximately 20 ml of dry benzene was heated to reflux and a few milligrams of cuprous chloride were placed in the flask. Slow addition of an equimolar quantity (2.63 g) of ethyl diazoacetate in 10 ml benzene was begun and continued at such a rate as to allow for constant evolution of nitrogen. After being heated 8 hours, the dark brown solution was cooled, filtered, and evaporated to an oil which distilled at 222-260° at 0.11 mm Hg. This oil could be crystallized from methanol-water for a mp 38°. The yield was 6 g (58%) with η_D^{35} 1.5069. The absorption spectra was $\lambda_{max\ (m\mu)}^{ethanol\ (95\%)}$ 278s, 287, and 298s; $\lambda_{max\ (\mu)}^{neat}$ 5.78 (C=O) and 8.68 (ROC=O). No N-H band was observed.

Analysis: Calcd. for $C_{30}H_{49}NO_2$: N, 3.07%; Found: N, 3.05%.

1-Alkyl Indole-3-Acetic Acids

VI   VII
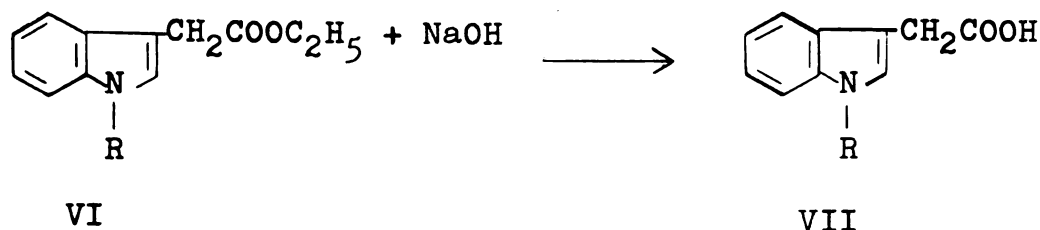
1-Methylindole-3-acetic acid (VII, R = methyl)

Five grams (0.023 mole) of ethyl 1-methylindole-3-acetic acid was saponified in 100 ml of approximately 2 N aqueous methanolic sodium hydroxide by refluxing for 6 hours. The solution was extracted with ether after evaporation of the solvent and re-addition of water. Neutralization of the aqueous phase was followed by extraction of the free acid

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Into ether. After the ether was dryed with anhydrous calcium chloride and evaporated, the yield was 3.7 g (85%) of a crystalline solid which was then recrystallized from cold methanol by addition of water. Recrystallization from benzene gave an analytical sample with mp 127-128°. Literature values were 127-128° (40) and 128° (4). Absorption spectra showed $\lambda_{max\ (m\mu)}^{ethanol\ (95\%)}$ 277s, 287 and 298s; $\lambda_{max\ (\mu)}^{KBr}$ 3-4 (COOH) and 5.89 (acid C=O).

1-Ethylindole-3-acetic acid (VII, R = ethyl)

One and four-tenths grams (0.0061 mole) of ethyl 1-methylindole-3-acetate was saponified by boiling the ester with 2 N solution of methanol-water (50:50). After 8 hours, the solvent was removed in vacuo and water was added. This solution was extracted twice with ethyl ether and neutralized with concentrated hydrochloric acid. The aqueous phase was re-extracted with ether to remove the product. The ether was washed, dryed, and evaporated. Then the acid was crystallized from methanol-water to yield 0.75 g (63%). Recrystallization from benzene-hexane gave a product melting at 107-108°. The literature gives a range of values: 102° (40), 106° (4). Infrared and ultraviolet spectra showed $\lambda_{max\ (\mu)}^{KBr}$ 3-4 (COOH), 5.89 (acid C=O); $\lambda_{max\ (m\mu)}^{ethanol\ (95\%)}$ 278s, 288, and 298s.

1-n-Propylindole-3-acetic acid (VII, R = n-propyl)

Three grams (0.0122 mole) of ethyl 1-n-propylindole-3-acetate dissolved in methanol to which an equal volume of

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4 $\underline{N}$ sodium hydroxide had been added was heated at reflux for 6 hours. Upon evaporation of the solvent, water was added and the solution extracted twice with ether. After neutralization of the aqueous solution with concentrated HCl, ether was used to extract the acid. This ether extract was washed twice with water, dried with anhydrous calcium chloride and evaporated to produce a residue which was crystallized from methanol-water to yield 1.65 g (63%) of product. Recrystallization from benzene-hexane gave a melting point of 80-81°. The literature value was 85° (4). Absorption data were $\lambda_{max (m\mu)}^{ethanol (95\%)}$ 278s, 288, and 298s; $\lambda_{max (\mu)}^{KBr}$ 3-4 (acid C=O), 5.87 (C=O).

1-$\underline{iso}$-Propylindole-3-acetic acid (VII, R = $\underline{iso}$-propyl)

Two normal sodium hydroxide in 50% methanol containing a suspension of 1 g (0.0041 mole) ethyl 1-$\underline{iso}$-propylindole-3-acetate was refluxed 6 hours and evaporated $\underline{in}$ $\underline{vacuo}$ and the residue was redissolved in water. After the solution was extracted twice with ethyl ether, it was neutralized with concentrated hydrochloric acid and reextracted with ether. Washing of the ether extract and drying with anhydrous calcium chloride was followed by evaporating the ether and dissolving the residue in methanol. Gradual addition of water to the cold methanol solution caused precipitation of 0.4 g (45%) of crystals, mp 104.5-105.5°. The melting point was not changed upon recrystallization from benzene-hexane. Absorption data were $\lambda_{max (m\mu)}^{ethanol (95\%)}$ 279s, 288, and 298s; $\lambda_{max (\mu)}^{KBr}$ 3-4 (COOH),

5.88 (acid C=O).

Analysis: Calcd. for $C_{13}H_{15}NO_2$: N, 6.45%; Found: N, 6.46%.

1-n-Butylindole-3-acetic acid (VII, R = n-butyl)

Saponification of 2 g (0.077 mole) ethyl 1-n-butyl-indole-3-acetate with 2 N sodium hydroxide in 50% methanol for 6 hours at reflux gave the title acid in 78% yield (1.4 g). After the solvent was evaporated and water was added, the basic solution was extracted with ethyl ether, which was then washed with water. When the ether had been dried with anhydrous calcium chloride, it was evaporated to a residue which was crystallized from cold methanol by careful addition of water. Recrystallization of this material from benzene-hexane mixture gave an analytical sample with melting point 75-76°. Absorption showed $\lambda_{max\ (m\mu)}^{ethanol\ (95\%)}$ 278s, 288, and 298s; $\lambda_{max\ (\mu)}^{KBr}$ 3-4 (COOH), 5.88 (acid C=O).

Analysis: Calcd. for $C_{14}H_{17}NO_2$: N, 6.06%; Found: N, 6.10%.

1-iso-Butylindole-3-acetic acid (VII, R = iso-butyl)

A methanol solution of 1.38 (0.0053 mole) ethyl 1-iso-butylindole-3-acetate was treated with an equal volume of 4 N sodium hydroxide and refluxed 6 hours. The solvent was removed in vacuo and water was added again. Extraction of the aqueous solution with ethyl ether was followed by neutralization with concentrated hydrochloric acid and ether extraction of the product. The ether was evaporated and the

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residue was crystallized from methanol-water giving 0.7 g (57%) material with a melting point of 93-94°, which was not improved by recrystallization from benzene-hexane. The compound absorbed infrared and ultraviolet radiation as follows: λ_{max}^{KBr} (μ) 3-4 (COOH), 5.87 (acid C=O); $\lambda_{max\ (m\mu)}^{ethanol\ (95\%)}$ 278s, 288, and 298s.

Analysis: Calcd. for $C_{14}H_{17}NO_2$: N, 6.06%; Found: N, 5.98%.

1-sec-Butylindole-3-acetic acid (VII, R = sec-butyl)

Saponification of a methanol solution of 1.4 g (0.0054 mole) ethyl 1-sec-butylindole-3-acetate was effected by refluxing it with an equal volume 4 N sodium hydroxide for 6 hours. Evaporation in vacuo and readdition of water produced a solution which was then extracted with ethyl ether, neutralized with concentrated hydrochloric acid, and re-extracted with ether. The latter extract was dryed with anhydrous calcium chloride and evaporated to a mass which was crystallized from methanol-water and recrystallized from benzene-hexane for a yield of 0.75 g (60%). Its melting point was 64-64.5°, and it showed absorption as follows: $\lambda_{max\ (m\mu)}^{ethanol\ (95\%)}$ 278s, 288, and 298s; λ_{max}^{KBr} (μ) 3-4 (COOH), 5.87 (acid C=O).

Analysis: Calcd. for $C_{14}H_{17}NO_2$: N, 6.06%; Found: N, 6.02%.

1-tert-Butylindole-3-acetic acid (VII, R = tert-butyl)

Ethyl 1-tert-butylindole-3-acetate (1.4 g, 0.0054 mole) was dissolved in methanol and an equal volume of 4 N

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sodium hydroxide added. The solution was refluxed 6 hours and evaporated. Water was added and the solution extracted twice with ethyl ether. Neutralization of the aqueous extract with concentrated hydrochloric acid was followed by extraction of the product with ether. Evaporation of the solvent gave a residue which was crystallized from methanol by the addition of water for a yield of 0.3 g (25%). The slightly green crystals were recrystallized from the same solvent system by using Norit A charcoal. Final crystallization from benzene-hexane gave white crystals with mp 104.5-105.5°. Ultraviolet and infrared absorption were: $\lambda_{max (m\mu)}^{ethanol}$ (95%) 276s, 286, and 295s; $\lambda_{max (\mu)}^{KBr}$ 3-4 (COOH) 5.87 (acid C=O).

Analysis: Calcd. for $C_{14}H_{17}NO_2$: N, 6.06%; Found: N, 6.27%.

1-n-Pentylindole-3-acetic acid (VII, R = n-pentyl)

Saponification in 2 N sodium hydroxide aqueous methanol (50%) of 2 g (0.0073 mole) of ethyl 1-n-pentylindole-3-acetate by refluxing for 6 hours gave the corresponding acid in 81% yield (1.46 g). Isolation of the acid was effected by removing the solvent from the basic mixture, redissolving the salt in water, extracting with ethyl ether to remove neutral impurities, and finally neutralizing to free the acid. Ether extraction of this solution followed by evaporation of the ether gave a solid which was crystallized (Norit A) from methanol-water. The melting point was

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63-64°. Absorption data were: $\lambda^{\text{ethanol }(95\%)}_{\text{max (m}\mu)}$ 278s, 288, and 298s; $\lambda^{\text{KBr}}_{\text{max }(\mu)}$ 3-4 (COOH) 5.87 (acid C=O).

Analysis: Calcd. for $C_{15}H_{19}NO_2$: N, 5.71%; Found: N, 5.66%.

1-n-Decylindole-3-acetic acid (VII, R = n-decyl)

Refluxing a methanol solution of 2.3 g (0.0067 mole) ethyl 1-n-decylindole-3-acetate with an equal volume of 4 N sodium hydroxide for 6 hours resulted in saponification of the ester. After the solvent was removed in vacuo, water was added. Extraction with ethyl ether was followed by neutralization with concentrated hydrochloric acid and re-extraction with ether to remove the product. Removal of the solvent gave a solid (1.75 g, 83%) which was crystal-lized from methanol and water. It was recrystallized from benzene and hexane to give a product with mp 51-51.5°. The absorption spectra showed $\lambda^{\text{ethanol }(95\%)}_{\text{max (m}\mu)}$ 278s, 288, and 298s; $\lambda^{\text{KBr}}_{\text{max }(\mu)}$ 3-4 (COOH) and 5.88 (acid C=O).

Analysis: Calcd. for $C_{20}H_{29}NO_2$: N, 4.44%; Found: N, 4.44%.

1-n-Octadecylindole-3-acetic acid (VII, R = n-octadecyl)

Saponification of 4 g (0.0093 mole) ethyl 1-n-octa-decylindole-3-acetate was effected by refluxing a methanolic solution of the ester in a like volume of 4 N sodium hydrox-ide. After removal of the solvent in vacuo and readdition of water, the basic solution was extracted with ethyl ether. Neutralization with concentrated hydrochloric acid was

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followed by extraction of the carboxylic acid with ether.

The ether was washed and evaporated to give 3.35 g (90%) of

a solid which was crystallized three times from methanol.

Norit A was used on the final crystallization. Melting

point was 75.5-76.5° with absorption as follows: $\lambda_{max (m\mu)}^{ethanol (95\%)}$

278s, 288, and 298s; $\lambda_{max (\mu)}^{KBr}$ 3-4 (COOH) and 5.85 (acid C=O).

Analysis: Calcd. for $C_{28}H_{45}NO_2$: N, 3.28%; Found:

N, 3.31%.

Ethyl Indole-3-Acetate

Seventy-five grams of crude indole-3-acetic acid,

which had been prepared by the high temperature autoclave

procedure of Johnson and Crosby (89), was dissolved in 2 l

of absolute ethanol to which had been added 4 g of p-

toluenesulfonic acid. The solution was refluxed 16 hours

on an electric heating mantle and cooled, and the ethanol

was removed in vacuo. Ethyl ether was added to the residue

and the solution was extracted with dilute sodium bicarbon-

ate. After washing the ether layer with water and drying it

with magnesium sulfate, the solvent was removed by distilla-

tion. Vacuum distillation of the residual oil at 155° at

0.8 mm Hg afforded a clear liquid in 75.8% yield (65.8 g).

After standing in a refrigerator for several hours, the

material crystallized. Recrystallization from methanol and

water gave white needles mp 42-43°.

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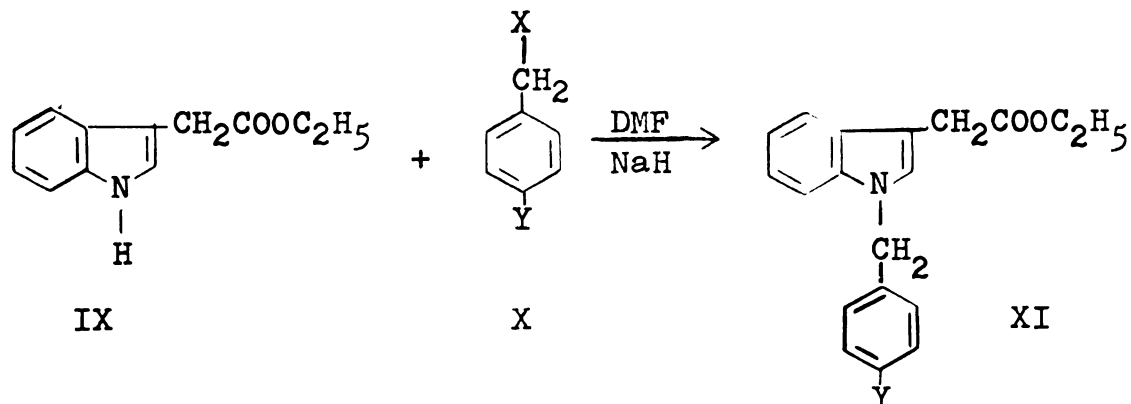
Ethyl 1-benzylindole-3-acetate (XI, Y = H)

Eight and fifty-four hundredths grams (0.042 mole) of ethyl indole-3-acetate dissolved in about 50 ml dry dimethylformamide was added slowly to a stirred dimethylformamide (109) suspension of 2.12 g (0.044 mole) sodium hydride (50% in oil). After the ester had been added, stirring was continued for 30 minutes. The solution was cooled to 0° and 5.31 g (0.042 mole) of benzyl chloride in approximately 50 ml of dimethylformamide was added slowly while the reaction mixture was stirred. The reaction mixture was allowed to warm to room temperature overnight and was then heated at 60° for 3 hours. After removal of the solvent in vacuo, the remaining oil was distilled at 170° and 0.1 mm Hg. Julia and Tchernoff (40) report 180-200° at 0.8 mm Hg but do not report a melting point. Careful addition of water to a methanol solution of the oil resulted in crystallization. The melting point was 44-44.5°. The yield was 4.55 g (39%).

A similar yield was obtained when the oil prepared as described above was subjected to preparative layer

chromatography rather than distillation. An analytical
sample was so prepared. Absorption spectra were $\lambda_{max}^{ethanol\ (95\%)}$ (mμ)
277s, 285, and 296; λ_{max}^{KBr} (μ) 5.78 (C=O), 8.70 (ROC=O).

Analysis: Calcd. for $C_{19}H_{19}NO_2$: N, 4.77%; Found:

N, 4.77%. It was then evident that a significant amount of

by-product, which had been isolated in the chromatographic

procedure, was present. This by-product was characterized

as the benzyl ester of indole-3-acetic acid through consider-

ation of its ultraviolet and infrared spectra.

Ethyl 1-p-fluorobenzylindole-3-acetate (XI, Y = F)

Ethyl indole-3-acetate (12.8 g, 0.063 mole) in approx-

imately 75 ml of dry dimethylformamide was added dropwise to

a stirred dimethylformamide suspension of 3.18 g (0.66 mole)

of 50% sodium hydride in oil. The mixture was stirred for

20 minutes and then was cooled to 0°. A dimethylformamide

solution of 9.64 g (0.067 mole) of p-fluorobenzyl chloride

was added slowly while the reaction mixture was stirred and

its temperature maintained at 0°. When addition of the

halide was complete, the mixture was allowed to warm to

room temperature and was stirred for 16 hours longer. After

chloroform and water were added and the chloroform was

removed, the aqueous solution was extracted a second time

with chloroform. The combined chloroform extracts were

washed extensively with water, dryed with anhydrous calcium

chloride, and evaporated to yield an oil. The oil distilled

at 170-180° and 0.01 mm Hg and yielded 8.5 g (44%). The oil

could be crystallized from methanol-water. However, in order
to get a pure product devoid of N-H stretch in the infrared
spectrum, it was necessary to purify the material on prepara-
tive layer chromatography. The melting point was 69°.
Absorption spectra showed $\lambda_{max\ (m\mu)}^{ethanol\ (95\%)}$ 267, 272, 285, and
295; $\lambda_{max\ (\mu)}^{KBr}$ 5.79 (C=O), 8.64 (ROC=O).

__Analysis__: Calcd. for $C_{19}H_{18}NO_2F$: N, 4.50%; Found:
N, 4.47%.

Ethyl __p__-chlorobenzylindole-3-acetate (XI, Y = Cl)
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To a stirred suspension of 1.06 g (0.022 mole) of
sodium hydride (50% in oil) in dry dimethylformamide was
added 4.26 g (0.021 mole) of ethyl indole-3-acetate. After
addition of the ester was completed, the mixture was stirred
for 20 minutes and cooled to the temperature of a dry ice-
acetone bath. Three and fifty-eight hundredths g (0.0222
mole) of __p__-chlorobenzyl chloride in approximately 50 ml
dimethylformamide was added dropwise to the cooled solution.
Stirring was continued as the solution was allowed to warm
overnight to room temperature. After water was added, the
solution was extracted twice with chloroform. The combined
chloroform extracts were washed well with water and dryed
with anhydrous calcium chloride. After the solvent was
evaporated, the residual oil was fractionally crystallized
from methanol-water. The first fractions were the title
compound which was present in 43% yield (3 g). Recrystal-
lization from methanol gave a product with improved color.

Preparative layer chromatography was employed for purification of the analytical sample. The sample melted at 96-98°. Absorption spectra were consistent with the assigned structure: $\lambda_{max (m\mu)}^{ethanol\ (95\%)}$ 277, 285, and 295; $\lambda_{max\ (\mu)}^{KBr}$ 5.79 (C=O), 8.60 (ROC=O).

Analysis: Calcd. for $C_{19}H_{18}NO_2Cl$: N, 4.27%; Found: N, 4.22%.

Ethyl 1-p-bromobenzylindole-3-acetate (XI, Y = Br)

One hundred six milligrams (0.0022 mole) of sodium hydride (50% in oil) was stirred in 10 ml dry dimethylformamide while 0.43 g (0.0021 mole) of ethyl indole-3-acetate was added dropwise. After the green solution was stirred for 20 minutes and then cooled to 0°, dropwise addition of 0.557 g (0.00222 mole) of p-bromobenzyl bromide was begun. Stirring was continued overnight as the solution warmed to room temperature. Water was added and the solution was extracted twice with ethyl ether. The ether solution was washed extensively with water and then dryed with anhydrous calcium chloride. Next the ether was removed **in vacuo** and a little methanol and water were added. After sitting in the cold for a day, 0.24 g (31%) of the product was isolated. Additional material could be obtained from the filtrate by preparative layer chromatography.

The absorption data were $\lambda_{max\ (m\mu)}^{ethanol\ (95\%)}$ 274, 283, and 296s; $\lambda_{max\ (\mu)}^{KBr}$ 5.79 (C=O), 8.45 (ROC=O). The melting point was 104-104.5°.

Analysis: Calcd. for $C_{19}H_{18}NO_2Br$: N, 3.76%; Found: N, 3.81%.

Ethyl 1-p-methylindole-3-acetate (XI, Y = CH_3)

To a stirred suspension of 0.106 g (0.0022 mole) of sodium hydride in 10 ml dry dimethylformamide was added 0.46 g (0.0021 mole) of ethyl indole-3-acetate in 10 ml dimethylformamide. After all the ester was added, the mixture was stirred for 20 minutes and then was cooled to $0°$. To the cooled solution 0.313 g (0.00222 mole) of p-methyl-benzyl chloride in 10 ml dimethylformamide was added slowly with stirring. The mixture was stirred and allowed to warm to room temperature overnight. Water was added and the product extracted with two portions of ethyl ether. The pooled ether extracts were washed several times with water, dried with anhydrous calcium chloride, and evaporated to give an oil. Five milliliters of acetone was added. One and one-fourth ml of this solution was streaked on a preparative layer chromotography plate and developed in 18% butanone-hexane. The product moved at a higher R_f (0.4) than did the chief by-product—an ester described below. Yield was 80 mg (41%). The melting point was $65.5°$. Absorption data included $\lambda_{max (m\mu)}^{ethanol}$ (95%) 275, 285, and 296s; $\lambda_{max (\mu)}^{KBr}$ 5.79 (C=O), 8.70 (ROC=O).

Analysis: Calcd. for $C_{20}H_{21}NO_2$: N, 4.56%; Found: N, 4.61%.

Ethyl 1-p-methoxybenzylindole-3-acetate (XI, Y = CH$_3$O)

Eight and fifty-four hundredths grams (0.042 mole) of ethyl indole-3-acetate in 100 ml dimethylformamide was added dropwise to a stirred dimethylformamide suspension of 2.12 g (0.1044 mole) sodium hydride (50% in oil). Having been stirred for 20 minutes, the solution was cooled to 0°. Seven grams (0.0444 mole) of p-methoxybenzyl chloride (which had been synthesized from anisyl alcohol and hydrogen chloride in benzene solution at -10°) in 50 ml dimethylformamide was added drop by drop while maintaining vigorous agitation. The mixture was allowed to stir for 24 hours as it warmed to room temperature. Water was added and the solution was extracted twice with chloroform. The chloroform extracts were pooled and washed freely with water. After drying the extract with anhydrous calcium chloride, the solvent was removed in vacuo to yield approximately 50% (6.1 g) of crude product. Attempts were then made to crystallize the residual oil. While it was not possible to effect crystallization at this point, a sample of the saponified material was later re-esterified and purified with preparative layer chromatography to yield the analytical sample with the melting point being 38.5-40°. The absorption showed $\lambda_{max (m\mu)}^{ethanol\ (95\%)}$ 278, 284, and 297s; $\lambda_{max (\mu)}^{KBr}$ 5.79 (C=O), 8.60 (ROC=O).

Analysis: Calcd. for C$_{20}$H$_{21}$NO$_3$: N, 4.33%; Found: N, 4.25%.

1-p-Substituted Benzylindole-3-Acetic Acids
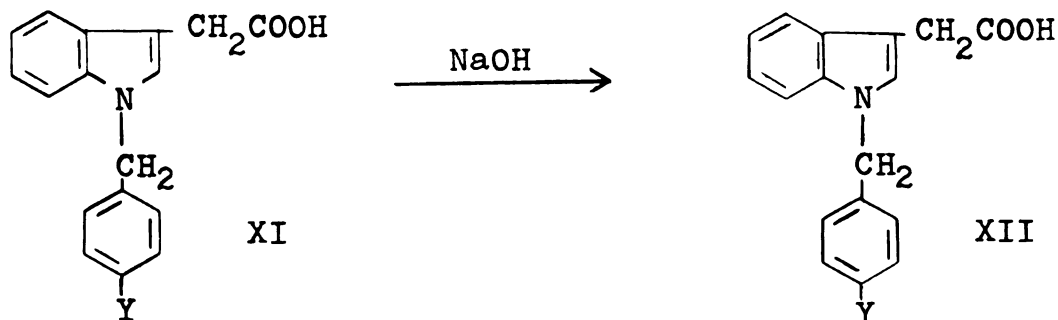
1-Benzylindole-3-acetic acid (XII, Y = H)

One gram (0.00341 mole) of crystalline ethyl 1-benzyl-indole-3-acetate was dissolved in approximately 25 ml of methanol. An equal volume of 19% sodium hydroxide was added and the mixture was refluxed for 6 hours. After the solvent was distilled in vacuo, the residue was taken up in methanol. Water was added to the salt and the basic solution extracted with ethyl ether. Hydrochloric acid was used to neutralize the aqueous extract, which was then extracted with ether. After the ether phase was washed with water and evaporated to dryness, the residue was dissolved in methanol. Water was added to precipitate the product as white crystals. mp 155-156°. Julia and Tchernoff (40) reported mp 149°. The ultraviolet and infrared spectra showed these features: $\lambda_{max \ (m\mu)}^{ethanol \ (95\%)}$ 277s, 286, and 296s; $\lambda_{max \ (\mu)}^{KBr}$ 3-4 (O-H), 5.88 (C=O).

1-p-Fluorobenzylindole-3-acetic acid (XII, Y = F)

One hundred milligrams (0.00032 mole) of ethyl p-

fluorobenzylindole-3-acetate, which had been purified with preparative layer chromatography, was treated with a 50% methanolic solution of 3.5 $\underline{N}$ sodium hydroxide. The solution was heated at reflux for 2 hours and then evaporated to dryness $\underline{in\ vacuo}$. Water was added to the residue and the solution extracted with ether. After the solution was neutralized with concentrated hydrochloric acid, the product was extracted with ether. The extract was washed twice with water and the ether was removed by distillation. Then the residue (39 mgs, 45%) was dissolved in methanol and water was added to the point of turbidity. Crystals formed after the flask was allowed to stand in the cold overnight. The melting point was 159.5-160.5°. Absorption of the compound was $\lambda_{max\ (m\mu)}^{ethanol\ (95\%)}$ 267, 273, 289, and 296s; $\lambda_{max\ (\mu)}^{KBr}$ 3-4 (O-H), 5.86 (C=O).

Analysis: Calcd. for $C_{17}H_{14}NO_2F$: N, 4.94%; Found: N, 4.96%.

1-p-Chlorobenzylindole-3-acetic acid (XII, Y = Cl)

A methanol solution of 135 mg (0.00041 mole) of ethyl 1-p-chlorobenzylindole-3-acetate, which had been purified with preparative layer chromatography, was combined with a like volume (10 ml) of 7 $\underline{N}$ sodium hydroxide. Saponification of the ester was effected by refluxing the solution 2 hours. After the solvent was evaporated, the residue was dissolved in water and the solution extracted with ethyl ether. Hydrochloric acid was employed to neutralize the base and the

product was then extracted into ethyl ether. The product

crystallized from benzene diluted with hexane. The yield

was 106 mg (85%) of white crystals, mp 145.5-147.5°.

Absorption data of the compound showed $\lambda_{max\ (m\mu)}^{ethanol\ (95\%)}$ 277,

285, and 295s; $\lambda_{max\ (\mu)}^{KBr}$ 3-4 (acid OH), 5.88 (C=O).

Analysis: Calcd. for $C_{17}H_{14}NO_2Cl$: N, 4.67%; Found:

N, 4.68%.

1-p-Bromobenzylindole-3-acetic acid (XII, Y = Br)

Ten milliliters of 7 N sodium hydroxide was added to

an equivalent volume of methanol containing 35 mg (0.000094

mole) of ethyl 1-p-bromobenzylindole-3-acetate which had

been purified previously with preparative layer chromato-

graphy. After the solution was heated at reflux for 2 hours

and evaporated to dryness, water was added to the residue

and the basic solution extracted twice with ethyl ether.

Concentrated hydrochloric acid was used to neutralize the

solution, which was then extracted with ether. The ether

was washed with water, dryed with anhydrous calcium chloride,

and removed from the residue by distillation in vacuo. The

product crystallized from benzene in a yield of 17 mg (54%).

The absorption was $\lambda_{max\ (m\mu)}^{ethanol\ (95\%)}$ 277, 285, 295; $\lambda_{max\ (\mu)}^{KBr}$

3-4 (acid OH), 5.87 (C=O), and a small band at 2.9 (H_2O or

N-H). The melting point was 152°.

Analysis: Calcd. for $C_{17}H_{14}NO_2Br$: N, 4.07%; Found:

N, 4.06%.

1-p-Methylbenzylindole-3-acetic acid (XII, Y = CH$_3$)

After ethyl 1-p-methylbenzylindole-3-acetate (90 mg) was dissolved in 10 ml methanol, 10 ml $\underline{N}$ sodium hydroxide was added. The solution was refluxed 2 hours then evaporated to dryness. Water was added and the solution was extracted with ethyl ether. The aqueous solution was neutralized with concentrated hydrochloric acid and extracted with ethyl ether. After the ether extract was washed with water and dryed with anhydrous calcium chloride, the ether was removed by distillation. Benzene served as a useful solvent for crystallization. The melting point was 125-126°. A yield of 56 mg (68%) was obtained with absorption data as follows: $\lambda_{max\ (m\mu)}^{ethanol\ (95\%)}$ 274, 286, 296s; $\lambda_{max\ (\mu)}^{KBr}$ 3-4 (acid-OH), 5.86 (C=O).

Analysis: Calcd. for C$_{18}$H$_{17}$NO$_2$: N, 5.01%; Found: N, 5.00%.

1-p-Methoxybenzylindole-3-acetic acid (XII, Y = CH$_3$O)

First six grams (0.0176 mole) of crude ethyl 1-p-methoxyindole-3-acetate was dissolved in methanol; an equal volume of 4 $\underline{N}$ sodium hydroxide was then added. The solution was refluxed 6 hours and evaporated to dryness $\underline{in\ vacuo}$. Water was added and the solution was extracted with ethyl ether. After the alkaline solution was neutralized with concentrated hydrochloric acid, the product was extracted with ether. Distillation of the ether $\underline{in\ vacuo}$ yielded a residue which was crystallized from methanol and water.

Recrystallization of the product from methanol and finally

from benzene gave the analytical sample in 51% yield (2.8 g).

The compound melted at 136-137°. The following absorptivity

was noted: $\lambda_{max\ (m\mu)}^{ethanol}{}^{(95\%)}$ 278, 284 and 296s; $\lambda_{max\ (\mu)}^{KBr}$ 3-4

(acid OH), 5.87 (C=O).

Analysis: Calcd. for $C_{18}H_{17}NO_3$: N, 4.74%; Found:

N, 4.85%.

1-(2',3',4',6'-Tetra-O-Acetyl-β-D-Glucopyranosyl) Indoline (XIV)
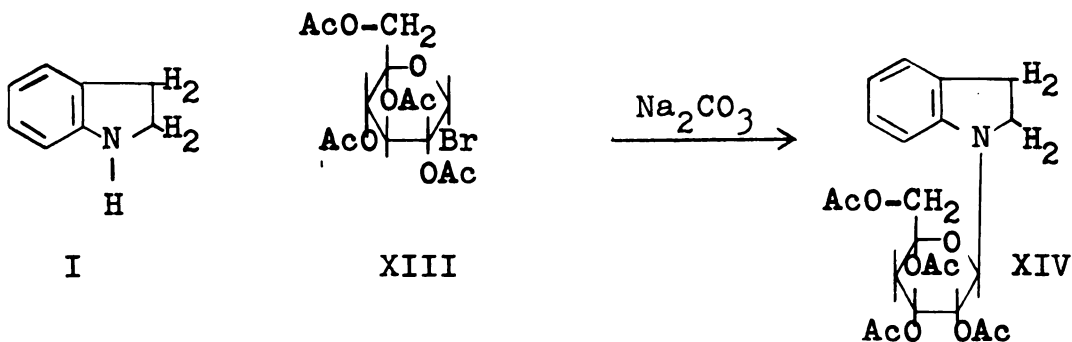
Three grams (0.025 mole) of indoline was reacted with

10.3 g (0.025 mole) of 1-(2,3,4,6-tetra-O-acetyl-α-D-

glucopyranosyl) bromide in the presence of 2.6 g (0.025 mole)

anhydrous, powdered sodium carbonate at 0°. Anhydrous ethyl

ether was added to make a slurry and this mixture was stirred

while it warmed to room temperature. After the solution was

refluxed for 3 days, water was added to the mixture and the

product was extracted twice with ether. Then the organic

layer was washed twice with water and dryed over anhydrous

magnesium sulfate. Removal of the solvent in vacuo was

followed by crystallization of the product from 95% ethanol.

Thin layer chromatography on Eastman sheet 6060 showed a

single spot with R_f 0.39 when developed in 25% butanone-hexane. The dimethylaminocinnamaldehyde spray reagent gave a red spot on the chromatogram. The yield was 7.38 g (66%) with a melting point of 117-118°, $[\alpha]_D^{25}$ 4.0° ($CHCl_3$, C=1).[1] The literature (98) gives 117.8-118.5° and $[\alpha]_D^{20}$ 5.5° (CCl_4).

1-(2',3',4',6'-Tetra-O-Acetyl-β-Glucopyranosyl)

Indole (XV)
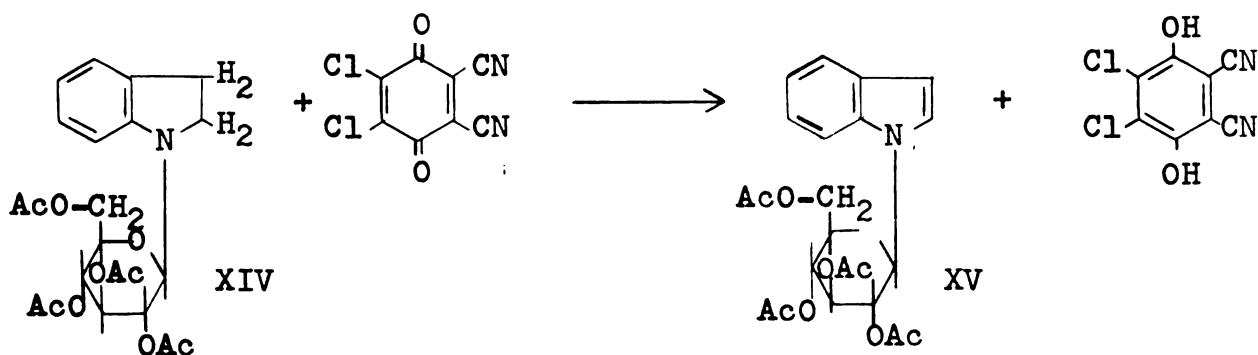
1-(2',3',4',6'-Tetra-O-acetyl-β-D-glucopyranosyl) indoline (7.35 g, 0.016 mole) was dehydrogenated with 3.72 g (0.0164 mole) dichlorodicyanobenzoquinone (DDQ) in a solution of 150 ml xylene by refluxing for 6 hours. This solution soon lost the dark red-violet color, and the substituted hydroquinone by-product precipitated. After filtering the warm solution, the solvent was removed in vacuo and the residue crystallized from methanol. This material moved as one spot at R_f 0.26-0.28 in 25% butanone-hexane on Eastman sheet 6060 thin-layer chromatography. The dimethylamino-cinnamaldehyde reagent slowly turned a blue color. Yield was 5.85 g (79%); melting point 148-149.5°. The literature value

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[1]Optical rotations were determined by using a Bendix Automatic Digital Polarimeter.

for the melting point was 148.5-149.5° (98). The optical

rotation was found to be $[\alpha]_D^{25}$ 1.3° (CHCl$_3$, C=1). The

reported optical rotation was $[\alpha]_D^{20}$ 1.5° (CHCl$_3$).

Ethyl 1-(2',3',4',6'-Tetra-O-Acetyl-β-D-Glucopyranosyl)-3-Indole Acetate (XVI)
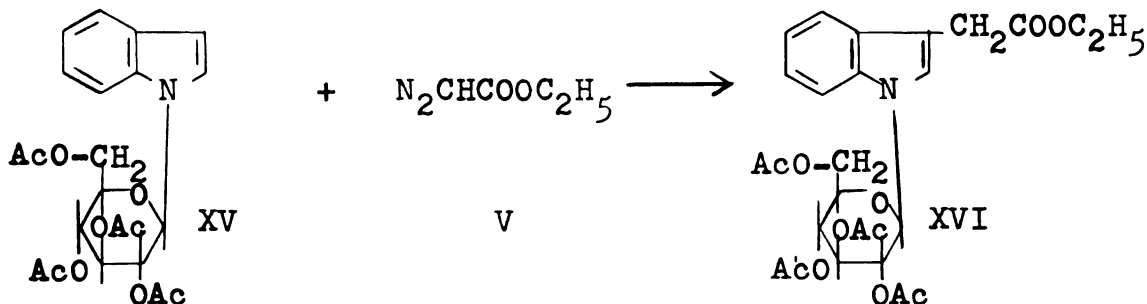
After a solution of 0.85 g (0.0019 mole) 1-(2',3',4', 6'-tetra-O-acetylglucopyranosyl)-indole in 10 ml xylene was brought to reflux on a wax bath, a catalytic amount of anhydrous cuprous chloride was added. To the refluxing solution was added dropwise a solution of 1.2-2.5 g (0.0073-0.02 mole) ethyl diazoacetate (V) in 10 ml xylene. Addition was continued at such a rate as to allow slow evolution of the nitrogen gas which formed. When addition of ethyl diazoacetate was completed, the reaction mixture was allowed to reflux for 8-12 hours. The dark yellow solution was cooled and the solvent was evaporated, leaving an oil. Purification of the product was effected by chromatography on a 3" x 4.5" column of silicic acid powder (Mallinckrodt 2844). After silicic acid was added to the residue along with acetone to make a slurry, the solvent was evaporated to yield a powder containing the product. This was layered on the

column, then eluted with 30% butanone-hexane. Fractions of
approximately 13 ml were collected and the product was found
in tubes 95-116. Then the eluate from tubes 96-106 was
pooled and evaporated _in vacuo_, and methanol was added.
After distillation and readdition of methanol, the product
crystallized when left for 3 hours at -15°. The yield of
crystalline material from tubes 96-106 was approximately
280 mg. An additional crop was obtained from the filtrate
of the first crop as well as from the fractions following
tube 106 by addition of water. Total yield was 40% (395
mg). The R_f was 0.20-0.23 in 25% butanone-hexane on
Eastman 6060 sheet thin-layer chromatography. The material
slowly gave a magenta spot when sprayed with the dimethyl-
aminocinnamaldehyde reagent. The melting point was 138-139°
with $[\alpha]_D^{25}$ -9.8 (CHCl$_3$, C=1). Absorption data were:
λ_{max}^{KBr} (μ) 5.74 (C=O), 7.95-8.25 (ROC=O); $\lambda_{max\ (m\mu)}^{ethanol\ (95\%)}$ 267,
279, and 290.

Analysis: Calcd. for $C_{26}H_{31}NO_{11}$: C, 58.53%;
H, 5.856%; N, 2.625%; Found: C, 58.19%; H, 5.83%; N, 2.87%.

Methyl 1-(β-D-Glucopyranosyl) Indole-3-Acetate (XVII)
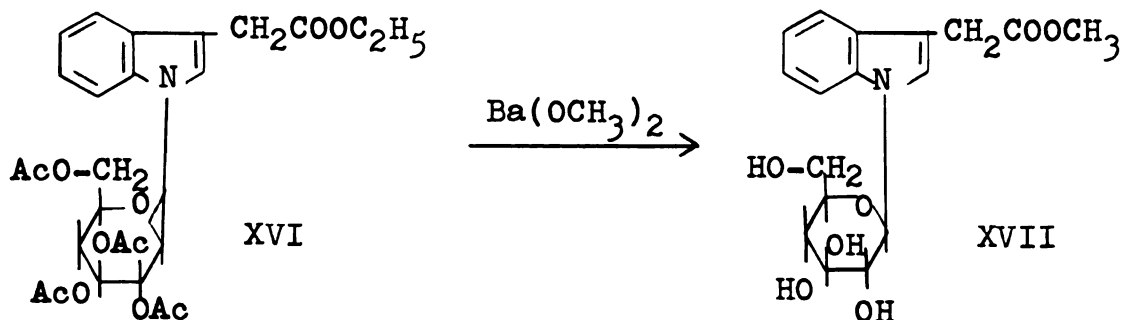
To an ice cold solution of 250 mg ethyl 1-(2',3',4',
6'-tetra-0-acetyl-β-D-glucopyranosyl)indole-3-acetate in
25 ml methanol was added 3.2 ml of 0.04 $\underline{N}$ barium methoxide
solution. The solution was allowed to stand 24 hours at
5°. Approximately 100 mg of damp IR 120 Amberlite resin
was added and then the mixture was stirred for 1 hour and
filtered. After evaporation and readdition of methanol,
the product crystallized on standing at -5° for 1 to 2 days.
A slowly developing magenta spot appeared when treated with
dimethylaminocinnamaldehyde. The R_f was 0.15 using Eastman
sheet 6060 thin layer chromatography: $HCCl_3$:EtOAc:HCO_2H,
5:5:1. Yield was 110 mg, (67%). The melting point when
the material was crystallized from methanol was 163-164°.
Absorption data were: λ_{max}^{KBr} (μ) 2.75-3.2 (OH), 5.83 (ester
C=O), 8.1-8.55 (ROC=O); $\lambda_{max}^{ethanol\ (95\%)}$ (mμ) 272, 279, and 290.
The optical rotation was $[\alpha]_D^{25}$ -8.3° (CH_3OH, C=1).

Analysis: Calcd. for $C_{17}H_{21}NO_7$: C, 58.11%,
H, 6.024%; N, 3.986%. Found: C, 57.73%; H, 6.17%;
N, 3.96%.

1-p-Substituted Benzyl Indolines and Indoles
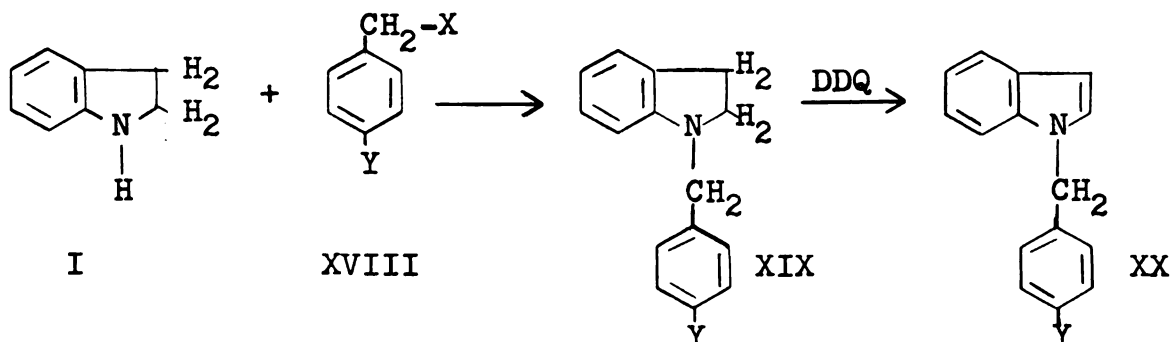
1-p-Chlorobenzylindoline (XIX, Y = Cl)

p-Chlorobenzyl chloride (16.1 g, 0.1 mole) was added dropwise with stirring to a slurry containing 5.83 g (0.055 mole) of anhydrous, powdered sodium carbonate and 11.9 g (0.1 mole) of indoline in 100 ml of xylene at $0°$. The mixture was stirred overnight and then heated to $100°$ for about 0.5 hours. The solvent and a small amount of other distillable material was removed *in vacuo*. Distillation of the product was not possible because a thixotropic material formed in the condenser. Therefore, the residue was dissolved in ethyl ether and extracted twice with 1 N hydrochloric acid. Neutralization of the acidic solution with 7 N sodium hydroxide, extraction with ether, and evaporation of the solvent gave an oil which could not be crystallized from methanol and water. Reaction of the residue with excess benzene-sulfonyl chloride in base freed the mixture of unsubstituted indoline. After extraction of the basic solution with ether, the product was extracted from the ether layer with 1 N hydrochloric acid. Neutralization of the acid fraction and re-extraction with ether gave a product which crystallized from methanol-water upon standing in a refrigerator overnight. The yield was 1.73 g (7.2%) of crystals mp $26-27°$. Absorption data were consistent with the assigned structure: $\lambda^{ethanol\ (95\%)}_{max\ (m\mu)}$ 253 and 295; $\lambda^{neat}_{max\ (\mu)}$ 3.3, (C-H).

Analysis: Calcd. for $C_{15}H_{14}NCl$: N, 5.75%; Found: N, 5.91%.

1-p-Chlorobenzylindole (XX, Y = Cl)

A xylene solution of 1.5 g (0.0062 mole) of 1-p-chlorobenzylindoline was reacted with 1.4 g (0.0062 mole) of dichlorodicyanobenzoquinone. When the quinone was completely dissolved, the solution was heated to reflux temperature and maintained there for 5 hours. Filtration of the product was followed by evaporation of the solvent in vacuo to yield 0.95 g (64%) of crude oil. Crystallization of the oil from methanol-water was unsuccessful and hence, the product was used directly for the next synthetic step. An analytical sample was purified by preparative layer chromatography. The absorption of the compound was as follows: $\lambda_{max\ (m\mu)}^{ethanol\ (95\%)}$ 270 and 292; $\lambda_{max\ (\mu)}^{neat}$ 3.3 (C-H).

Analysis: Calcd. for $C_{15}H_{12}NCl$: N, 5.80%; Found: N, 5.66%.

1-p-Nitrobenzylindoline (XIX, Y = NO$_2$)

An ethyl ether solution of 2.16 g (0.01 mole) of p-nitrobenzyl bromide and 1.19 g (0.01 mole) of indoline was stirred together with 1.06 g (0.01 mole) of anhydrous, powdered sodium carbonate at 0°. The stirred ether solution was permitted to warm to room temperature overnight and was then extracted with water. After drying of the ethereal solution over anhydrous calcium chloride, it was filtered and concentrated to give a crystalline mass. The product was dissolved in hot methanol and upon cooling yielded 2 g (79%) of orange colored crystals mp 98.5-99.5°. The ultraviolet spectra was not typical of an indoline and showed

$\lambda^{ethanol}_{max\ (m\mu)}$ (95%) 258. The infrared data were: λ^{melt}_{max} $_{(\mu)}$ 6.6, 7.42 (NO$_2$).

Analysis: Calcd. for $C_{15}H_{14}N_2O_2$: N, 11.02%; Found: N, 10.95%.

1-p-Nitrobenzylindole (XX, Y = NO$_2$)

Four grams (0.0157 mole) of 1-p-nitrobenzylindoline was dissolved in 150 ml of dry xylene. An equal molar quantity of dichlorodicyanobenzoquinone (3.55 g) was added to the solution. When solution was complete, it was heated to the boiling point and refluxed for 6 hours. The solution was cooled and filtered.

The xylene was removed _in vacuo_ and the residue was crystallized from methanol. The yield was 1.95 g (49%) of crude product. Recrystallization from methanol gave an analytical sample with mp 102°. The observed absorption values were: $\lambda^{ethanol}_{max\ (m\mu)}$ (95%) 269 and 292; λ^{melt}_{max} $_{(\mu)}$ 6.6, 7.42 (NO$_2$).

Analysis: Calcd. for $C_{15}H_{12}N_2O_2$: N, 11.11%; Found: N, 11.10%.

Ethyl 1-p-Nitrobenzylindole-3-Acetate (XXI)
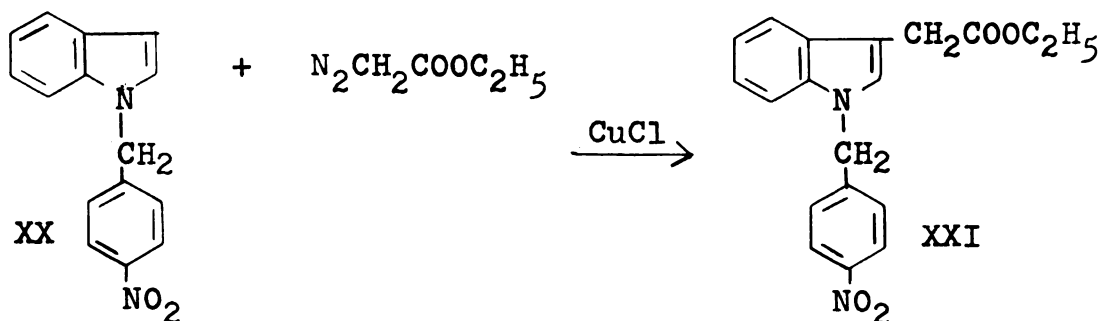
One and three-fourths grams (0.0067 mole) of 1-p-nitrobenzylindole was dissolved in about 20 ml of dry benzene and the solution heated to boiling. A few milligrams of anhydrous cuprous chloride was added, and addition of 1.53 g (0.0134 mole) of ethyl diazoacetate in a benzene solution was then begun at such a rate as to allow for smooth evolution of nitrogen. After all the ester had been added, heating was maintained for an additional 4 hours. The catalyst was removed by filtration and the solvent by distillation. The residue was dissolved in acetone and a few grams of the silicic gel (Mallinckrodt 2844) was added. After removal of the solvent, the residue was placed on top of a silica gel adsorbent in a 3 inch (diameter) by 4 inch (length) column. Elution of the adsorbent in the column with 30% butanone-hexane gave a peak of indolic material which moved at a lower R_f on thin layer chromatography than did the starting material. All tubes containing the indolic substance were pooled and the solvent concentrated in vacuo. The substance crystallized from methanol-water upon standing for several days in the cold. The yield of the product was 88 mg (4%) and had a mp of 101°. The following absorbancy properties were shown: $\lambda_{max}^{ethanol\ (95\%)}$ (mμ) 273 and 295; λ_{max}^{KBr} (μ) 5.79 (C=O) and 8.47 (ROC=O). A small band around 2.8 to 3.1 μ nearly disappeared after the sample had been melted thus indicating the presence of moisture.

Analysis: Calcd. for $C_{19}H_{18}N_2O_4$: N, 8.28%; Found: N, 8.01.

p-Substituted Benzyl Indole-3-Acetates

Several by-products of the reaction of a p-substituted benzyl halide with the N-sodium salt of ethyl indole-3-acetate were characterized. Elemental analysis and spectral data showed these to be the corresponding benzyl ester of indole-3-acetic acid. Yields were varible but approached that of the main product as determined by the intensity of the band on the preparative layer chromatography plate. Spraying the chromatogram with the dimethylaminocinnamaldehyde reagent[2] immediately gave a blue spot in each case. The by-product of each reaction moved at a lower R_f (0.30-0.35) than did the 1-substituted ester.

p-Methylbenzyl indole-3-acetate (XIII, Y = CH₃)

The major by-product was isolated by preparative layer chromatography from the reaction mixture of p-methylbenzyl chloride and the N-sodium salt of ethyl indole-3-acetate. This material was found to be the title compound from consideration of the following data: $\lambda_{max}^{ethanol\ (95\%)}$ (mµ) 273, 280, and 290; λ_{max}^{KBr} (µ) 2.99 (N-H), 5.81 (ester C=O), 8.55 (ROC=O). A melting point of 70-71° was found.

Analysis: Calcd. for $C_{18}H_{17}NO_2$: N, 5.01%; Found: N, 4.87%.

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[2]Dimethylaminocinnamaldehyde reagent was made from 50 ml 6 N HCl, 50 ml 95% ethanol, and 1 g dimethylaminocinnamaldehyde.

p-Chlorobenzyl indole-3-acetate (XIII, Y = Cl)

At $0°$ the reaction of p-chlorobenzyl chloride with sodium hydride and ethyl indole-3-acetate was unpredictable. In certain attempts only the title compound could be iso-lated from the reaction mixture while under apparently similar conditions a mixture of this compound and 1-p-chloro-benzylindole-3-acetate was isolated. In the latter case, this mixture was separated with preparative layer chromato-graphy and the lower band was eluted and recrystallized from methanol-water. The structure was indicated by these data: $\lambda_{max\ (m\mu)}^{ethanol\ (95\%)}$ 279 and 290; $\lambda_{max\ (\mu)}^{KBr}$ 2.94 (N-H), 5.79 (ester C=O), 8.65 (ROC=O); mp $95.5°$.

Analysis: Calcd. for $C_{17}H_{14}NO_2Cl$: N, 4.67%; Found: N, 4.67%.

p-Bromobenzyl indole-3-acetate (XIII, Y = Br)

The products of the reaction of p-bromobenzyl bromide with the N-sodium salt of ethyl indole-3-acetate were separated by preparative layer chromatography. The lower band was isolated and shown to have the following properties which characterized the structure: $\lambda_{max\ (m\mu)}^{ethanol\ (95\%)}$ 273, 280, and 290; $\lambda_{max\ (\mu)}^{KBr}$ 2.94 (N-HO), 5.79 (ester C=O), 8.64 (ROC=O); mp $102.5-104.°$

Analysis: Calcd. for $C_{17}H_{14}NO_2Br$: N, 4.07%; Found: N, 4.28%.

p-Nitrobenzyl indole-3-acetate (XIII, Y = NO₂)

A dimethylformamide slurry of 1.06 g (0.022 mole) of

sodium hydride (50% in oil) was vigorously stirred while 4.27 g (0.021 mole) of ethyl indole-3-acetate was added. After 20 minutes, the solution was cooled with a dry ice-acetone bath and 4.75 g (0.0222 mole) p-nitrobenzyl bromide in approximately 30 ml of dimethylformamide was added dropwise. The mixture was stirred overnight and water was added. The aqueous suspension was extracted with ethyl ether, the ether dryed with anhydrous sodium sulfate, and the solvent distilled. After dissolving the residue in hot methanol, the solution was diluted with water and allowed to stand in the cold. The crystals which formed were recrystallized from methanol-water to yield 3.5 g (53%) and melted at 114°. The following data indicate that the compound is not the expected 1-substituted ethyl ester but rather the title compound. Ultraviolet and infrared data were: $\lambda_{max}^{ethanol\ (95\%)}$ (mμ) 270 and 289; λ_{max}^{KBr} (μ) 2.98 (N-H), 5.79 (ester C=O), 8.56 (ROC=O).

Analysis: Calcd. for $C_{17}H_{14}N_2O_4$: N, 9.03%; Found: N, 8.87%.

Characterization of Compounds

The infrared absorbancy of liquid compounds was determined as films (neat) on sodium chloride plates. Solid compounds were analyzed in the form of potassium bromide pellets made from 410 mg of potassium bromide and one to two mg of the sample. Each solid sample was thoroughly mixed with the salt in a dental amalgamator (Cresent Dental Manufacturing Company) using glass beads

as a muller. A Beckman pellet press under a force of 20,000 lbs. was used to form the pellet in vacuo. Determination of the spectra was done on a Beckman Model Ir-5 double beam recording spectrophotometer.

All samples were dissolved in 95% ethanol for ultra-violet spectral analysis. One centimeter Silica cuvettes were used in a Beckman Model DK-2 ratio-recording spectro-photometer.

Melting points, which were obtained on a Fisher-Johns apparatus, are reported uncorrected. The refractive indexes were reported on all liquid samples using an Abbé refracto-meter. Spang Microanalytical Laboratory of Ann Arbor, Michigan, and Micro-Tech Laboratory of Skokie, Illinois, performed the elemental analyses.

Biological Assays

Tomato ovary growth assay

Solutions: A sufficient quantity of each alkyl compound to make a 10^{-3} $\underline{M}$ solution was added to 5 ml of lanolin (Merck) and alternately heated on a steam bath and mixed with a Vortex Jr. tube shaker several times. One-half ml of this solution was added to 4.5 ml of lanolin and the above heating and shaking procedure was repeated to prepare a 10^{-4} $\underline{M}$ solution. This method was repeated for the other dilutions of 10^{-5} $\underline{M}$ and 10^{-6} $\underline{M}$.

The benzyl derivatives and the glucose derivatives were dissolved in absolute ethanol and appropriate volumes of the solutions were added to the tubes containing 5 ml

lanolin to make final concentrations of 10^{-3} $\underline{M}$, 10^{-4} $\underline{M}$,
10^{-5} $\underline{M}$, and 10^{-6} $\underline{M}$ in lanolin. The ethanol was removed by
alternately heating on a steam bath and shaking with a
Vortex Jr. mixer. All samples were stored in a refriger-
ator until ready for use.

Plant material: Two varieties of tomato plants
(Lycopersicum esculentum, Michigan-Ohio hybrid and WR-7)
were grown in the greenhouse. The first flower cluster of
each plant was prepared for bioassay by trimming back all
of the immature flowers except two, which were emasculated
before anthesis. The calyx was trimmed flush with the
surface of the ovary. Later flower clusters were treated
in a similar manner for replications.

Treatment: Lanolin paste containing various concen-
trations (10^{-3} $\underline{M}$ to 10^{-6} $\underline{M}$) of each compound was applied to
the ovary in such a manner as to assure complete coverage
and in sufficient quantity to fill the cavity formed by the
truncated calyx. The bioassays of the first replication
indicated that all of the compounds had biological activity
less than did IAA; and therefore, subsequent replications
were made with 10^{-3} $\underline{M}$ solutions. Ovary diameter was mea-
sured after 6 to 10 days and compounds exhibiting low
activity were evaluated by recording delay of absission
relative to the controls.

Buckwheat root inhibition assay

Solutions: All solutions were made up in 0.05%
Tween 80 solution in glass distilled water. A sufficient

quantity of each compound to make 100 ml of 10^{-4} M solution was dissolved by alternately heating gently on a steam bath and agitating vigorously with a sonifier (Branson S-125) until all the material was in solution. From this stock solution, dilutions were made just before treatment. Five ml of stock solution was placed in a test tube and 0.5 ml was removed for addition to the next tube in the series which contained 4.5 ml of glass distilled water. After mixing, the process was repeated for the remaining dilutions.

Plant material: Japanese buckwheat seeds (Fagopyrum esculentum) were planted approximately 400 per 10 inch culture dish on filter paper which overlay a perforated procelain plate. Distilled water was added to a level just below the seeds. The seeds were allowed to germinate at 25° in the dark for 24 hours.

Treatment: Ten seeds with radicals approximately 1-3 mm in length were selected and placed on filter paper in a Petri dish. To each dish was added 4.5 ml of the test solutions. The roots were allowed to grow in the dark at 25° for 48 hours. The mean length in millimeters was determined for each compound and each concentration. Four replications were made on each compound.

Cucumber seedling curvature assay

Solutions: Lanolin solutions of previous experiments were used for this assay employing only 10^{-3} M concentrations.

Plant material: Cucumber seeds (Cucumis sativa,

variety MSU 736) were soaked for 8 hours under cold running tap water in a large beaker covered with cheesecloth. These seeds were planted in moist vermiculite and allowed to germinate in the dark for 48 hours. The trays were then placed in the laboratory under a bank of fluorescent lights until the plants were erect and the cotyledons extended. The plants were removed from the vermiculite, the root system washed free from non-plant material, and the stems clamped carefully between $\frac{1}{2}$" x $\frac{1}{2}$" x 15" strips of wood, one of which had a kerf cut at 1" intervals to accept the stems. The roots were placed in distilled water and allowed to equilibrate 2 to 5 hours before treatment.

Treatment: A quantity of lanolin paste sufficient to form a drop approximately 2 mm in diameter was placed on one cotyledon 1 mm from the stem. In different replications, the front and back leaves were treated so as to cancel any phototropic effects of stray lighting. Four replications were made to determine the angle of curvature and its time course. A plastic goniometer was used to measure the angles of the upper surfaces of the cotyledons in degrees every 15 minutes after the beginning of the initial curvature. No response was noted in the control plants treated with lanolin.

Bean petiole abscission assay

Solutions: Lanolin paste solutions of the test compounds prepared as previously described under tomato ovary growth assay were used for this test. Only 10^{-3} M solutions

were employed for this assay.

Plant material: Bean plants (Phaseolus vulgaris, variety Contender) were planted four to a pot and allowed to grow in a greenhouse until the primary leaves were well formed. Before the plumule had emerged, two uniform plants were selected in each pot and the other two were cut out. Petioles were cut off both primary leaves about 1 cm from the stalk with a razor blade.

Treatment: The exposed stub was treated immediately after cutting with lanolin paste containing the compounds to be assayed. Enough paste was applied to cover just the severed area and overlap the edge slightly to form a cap. After several days, when the first petioles began to abscise, observations were made thrice daily--in the morning, mid-day, and evening. Abscission was indicated when the petiole fell off under the influence of a device employing a spring brass blade which deflected to exert a force of approximately 10 g when the blade was pressed downward against the severed petiole. Time of abscission was recorded to the nearest hour. Five replications involving 4 petioles each were made of this assay.

Avena straight growth assay

Solutions: Fifty ml of the Tween 80 solutions of each compound prepared as described for the buckwheat assay was treated with sufficient dipotassium phosphate (89.7 mg), citric acid monohydrate (51 mg), and sucrose (1.00 g) to make a pH 5 buffered solution. A stock solution of buffer

was made from 20 g sucrose, 1.794 g dipotassium phosphate, 1.019 g citric acid monohydrate, and 1 ml Tween 80 diluted to 1,000 ml with glass distilled water. This buffer was used to dilute the 10^{-4} $\underline{M}$ stock solutions to 10^{-5} $\underline{M}$, 10^{-6} $\underline{M}$, 10^{-7} $\underline{M}$, and 10^{-8} $\underline{M}$ concentrations. Buffer solution was used as the control.

Plant material: Oat seeds (Avena sativa, variety Torch) were placed in a vacuum flask under tap water in the dark. A vacuum was drawn on the flask with a water aspirator and released after a few minutes and then immediately repeated. After intervals of 30 minutes, 45 minutes, and 45 minutes, the process was repeated. The seeds were planted on moist vermiculite in rectangular glass dishes after the final rinse. After 24 hours in the dark, red light was allowed to shine on the seeds for 2 hours. Then they were lightly covered with vermiculite which was moistened slightly. After 48 additional hours in the dark, 4.5 mm sections were cut 3 or 4 mm below the tip. These sections were floated for 2 hours on a glass distilled water solution containing 1 mg magnesium sulfate monohydrate per liter. All operations except red light treatment were carried out in the dark or under green light.

Treatment: Ten coleptile sections were placed in each six-inch test tube which contained 1.8 ml of each solution to be assayed. Assays were made on five concentrations (10^{-4} $\underline{M}$, 10^{-5} $\underline{M}$, 10^{-6} $\underline{M}$, 10^{-7} $\underline{M}$, 10^{-8} $\underline{M}$) of each compound by placing the charged tubes in a drum rotating at 1 rpm. The

drum was placed in a dark incubator at 26°. After 22 hours,
the sections were removed and placed in a photographic,
enlarger (Federal Manufacturing and Engineering Corporation).
The image, enlarged 5X, was measured in millimeters. Two to
four replications were made of each experiment. The data for
each concentration were averaged and presented as percent of
control growth.

RESULTS AND DISCUSSION

RESULTS AND DISCUSSION

Physical methods

The melting points or boiling points, results of elemental analyses, absorption data, and other physical constants of the various 1-alkylindole-3-acetic acids are given in Table 4. Corresponding data are tabulated in Tables 1, 2, and 3 for the intermediates employed in synthesizing these acids.

Infrared absorption spectra of a representative series of compounds are shown in Figures 1, 2, 3, and 4. The spectra include that of indoline which is contrasted with that of 1-n-decylindoline (Figure 1). In the latter case, the most conspicuous feature is the lack of N-H stretching absorption near 2.9 μ. That substitution has occurred on the indolic nitrogen is apparent by comparing the spectrum of the substituted indole with indole itself in Figure 2. Likewise, the lack of N-H stretch in the spectra of ethyl 1-n-decylindole-3-acetate and 1-n-decyl-indole-3-acetic acid in Figures 3 and 4 is good evidence for N-substitution.

In the latter two cases, carbonyl stretching vibrations are evident in the infrared spectra. Absorption near 8.0 and 8.6 μ is expected of esters and can be attributed to the ROC=O grouping in the ester (11) (Figure 3). All normal saturated esters except acetates absorb in

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Table 1. Physical Data for 1-Alkyl-Indolines

Compound	Molecular Weight	Boiling Point and Pressure Degrees C and mm Hg	Refractive Index η_D^{25}	Absorption Maxima			Calcd. % N	Found % N
				uv^1 mμ	N-H μ	C-H^2 μ		
Indoline[3]	119.17	40-44 at 0.08	1.5915	241 292	2.98	3.53	11.75	---
1-Methyl-indoline	133.20	44 at 0.08	1.5670	249 297	---	3.59 3.41 3.52	10.52	---
1-Ethyl-indoline	147.22	108-110 at 13	1.5580	252 300	---	3.39 3.58 3.42	9.51	---
1-n-Propyl-indoline	160.25	50-54 at 0.1	1.5478	254 302	---	3.40 3.58 3.50	8.74	8.71
1-iso-Propyl-indoline	160.25	46-48 at 0.1	1.5501	254 302	---	3.39 3.42 3.52	8.74	8.59
1-n-Butyl-indoline	175.28	130-131 at 13	1.5407	254 302	---	3.42 3.50 3.58	7.99	7.79

1-iso-Butyl-indoline	175.28	58-60 at 0.1	1.5379	256, 305	---	3.40, 3.58, 3.50	7.99, 7.93
1-sec-Butyl-indoline	175.28	64-66 at 0.2, 126-127 at 13	1.5379	257, 308	---	3.40, 3.43, 3.50, 3.54	7.99, 7.88
1-tert-Butyl-indoline	175.28	125-127 at 13	1.5431	256, 299	---	3.39, 3.53	7.99, 7.97
1-n-Pentyl-indoline	189.30	75-81 at 0.02	1.5318	254, 302	---	3.43, 3.51, 3.56	7.40, 7.29
1-n-Decyl-indoline	259.44	132 at 0.3	1.5164	256, 305	---	3.44, 3.52	5.40, 5.51
1-n-Octadecyl-indoline	371.66	209 at 0.25, mp 33.5-34.5	n_D^{35} 1.4954	254, 300	---	3.44, 3.52	3.77, 3.94

[1] Solvent was 95% ethanol.

[2] Wavelength values are the most prominent C-H band(s) given in decreasing order of intensity. Spectra were measured as films on sodium chloride plates.

[3] See Appendix I for abbreviations and structures.

Table 2. Physical Data for 1-Alkyl-Indoles

Compound	Molecular Weight	Boiling Point and Pressure Degrees C and mm Hg	Refractive Index η_D^{25}	Absorption Maxima			Calcd. % N	Found % N
				uv^1 mµ	N-H µ	$C-H^2$ µ		
Indole	117.15	253-254 at 760	----	268s 272 278 288	2.92	3.38 3.30	11.96	---
1-Methyl-indole	131.18	58-65 at 0.6	1.5945	274 282 294	---	3.41 3.58 3.52	10.68	---
1-Ethyl-indole	145.21	62-65 at 0.08 114-120 at 13	1.5860	274 282 294	---	3.38 3.42	9.65	---
1-n-Propyl-indole	159.23	71-73 at 0.3 64 at 0.15	1.5704	276 282 294	---	3.39 3.44 3.50	8.80	---
1-iso-Propyl-indole	159.23	69 at 0.08	1.5755	276 283 293	---	3.39 3.42s	8.80	---
1-n-Butyl-indole	173.26	139-145 at 13	1.5628	275 282 294	---	3.40 3.44 3.51	8.08	---

1-iso-Butyl-indole	173.26	75 at 0.5	1.5626	276 283 294	----	3.40 3.50	8.08 7.91
1-sec-Butyl-indole	173.26	69 at 0.08	1.5636	273 282 294	----	3.39 3.41 3.49	8.08 7.70
1-tert-Butyl-indole	173.26	75 at 0.08	1.5708	274 281 292	----	3.38	8.08 7.97
1-n-Pentyl-indole	187.29	82-95 at 0.07-0.08	1.5537	273 283 294	----	3.44 3.51	7.48 7.41
1-n-Decyl-indole	257.43	136-143 at 0.1	1.5264	270 283 294	----	3.44 3.52	5.44 5.39
1-n-Octadecyl-indole	369.64	210-225 at 0.1 mp 33	n_D^{35} 1.5108	276 283 294	----	3.42 3.50	3.79 3.77

[1] Solvent was 95% ethanol.

[2] The values of the most prominent band(s) are given in decreasing order of intensity. Spectra were determined on films of sodium chloride plates.

Table 3. Physical Data for Ethyl 1-Alkyl-Indole-3-Acetates

Compound	Molecular Weight	Boiling Point and Pressure Degrees C and mm Hg	Refractive Index η_D^{25}	Absorption Maxima			Calcd. % N	Found % N
				uv^1 mμ	N-H μ	C=O^2 μ		
Ethyl Indole-3-acetate	203.25	155 at 0.8	---	274 280 290	2.93	5.80	6.88	---
Ethyl 1-methyl-indole-3-acetate	217.27	125-130 at 0.09	1.5580	277s 287 298s	---	5.80	6.45	---
Ethyl 1-ethyl-indole-3-acetate	231.29	129-140 at 0.1	1.5492	277s 288 297s	---	5.80	6.06	---
Ethyl 1-n-propylindole-3-acetate	245.32	130-140 at 0.12	1.5455	278s 288 298s	---	5.79	5.71	5.76
Ethyl 1-iso-propylindole-3-acetate	245.32	128-138 at 0.15	1.5462	277s 287 298s	---	5.79	5.71	5.67
Ethyl 1-n-butylindole-3-acetate	259.34	159-165 at 0.5	1.5384	278s 287 297s	---	5.79	5.40	5.59

Compound	M.W.	b.p. (°C at mm)	n_D	U.V. (nm)				
Ethyl 1-iso-butylindole-3-acetate	259.34	130–145 at 0.3	1.5390	277s 287 297s	----	5.79	5.40	5.54
Ethyl 1-sec-butylindole-3-acetate	259.34	135–145 at 0.1	1.5416	277s 288 298s	----	5.78	5.40	5.78
Ethyl 1-tert-butylindole-3-acetate	259.34	127–140 at 0.09	1.5431	277s 285 295s	----	5.78	5.40	5.55
Ethyl 1-n-pentylindole-3-acetate	273.36	140–160 at 0.09	1.5326	277s 288 298	----	5.79	5.12	5.21
Ethyl 1-n-decylindole-3-acetate	343.51	166–188 at 0.1	1.5164	277s 287 297s	----	5.79	4.08	4.19
Ethyl 1-n-octadecylindole-3-acetate	455.73	222–260 at 0.11 mp 38	n_D^{35} 1.5069	278s 288 298s	----	5.78	3.07	3.05

[1] Ultraviolet spectra were determined in 95% ethanol solutions.

[2] Infrared spectra were measured on films of sodium chloride plates.

Table 4. Physical Data for 1-Alkyl-Indole-3-Acetic Acids

Compound	Molecular Weight	Melting Point[1] Degrees C	Absorption Maxima[3]				
			uv[2] mμ	N-H μ	C=O μ	Calcd. % N	Found % N
Indole-3-acetic acid	175.18	165-168	274 280 290	2.96	5.90	8.00	---
1-Methylindole-3-acetic acid	189.22	127-128	277s 287 298s	---	5.89	7.04	---
1-Ethylindole-3-acetic acid	203.24	107-108	278s 288 298s	---	5.89	6.89	---
1-n-Propylindole-3-acetic acid	217.27	80-81	278s 288 298s	---	5.87	6.45	---
1-iso-Propylindole-3-acetic acid	217.27	104.5-105.5	279s 288 298s	---	5.88	6.45	6.46
1-n-Butylindole-3-acetic acid	231.30	75-76	278s 288 298s	---	5.88	6.06	6.10

1-iso-Butylindole-3-acetic acid	231.30	93-94	278 288 298s	---	5.87	6.06	5.98
1-sec-Butylindole-3-acetic acid	231.30	64-64.5	278s 288 298s	---	5.87	6.06	6.02
1-tert-Butylindole-3-acetic acid	231.30	104.5-105.5	276s 286 295s	---	5.87	6.06	6.27
1-n-Pentylindole-3-acetic acid	245.32	63-64	278s 288 298s	---	5.87	5.71	5.66
1-n-Decylindole-3-acetic acid	315.46	51-51.5	278s 288 298s	---	5.88	4.44	4.44
1-n-Octadecylindole-3-acetic acid	427.67	75.5-76.5	278s 288 298s	---	5.85	3.28	3.31

[1]Melting points were determined on a Fisher-Johns Apparatus and are uncorrected.

[2]Ultraviolet spectra were measured in 95% ethanol solutions.

[3]All of the substituted acids showed the typical broad acid band in the range 3.0 to 3.8 μ.

FIGURE 1

Infrared spectra for:
  Upper; Indoline
  Lower; n-Decylindoline
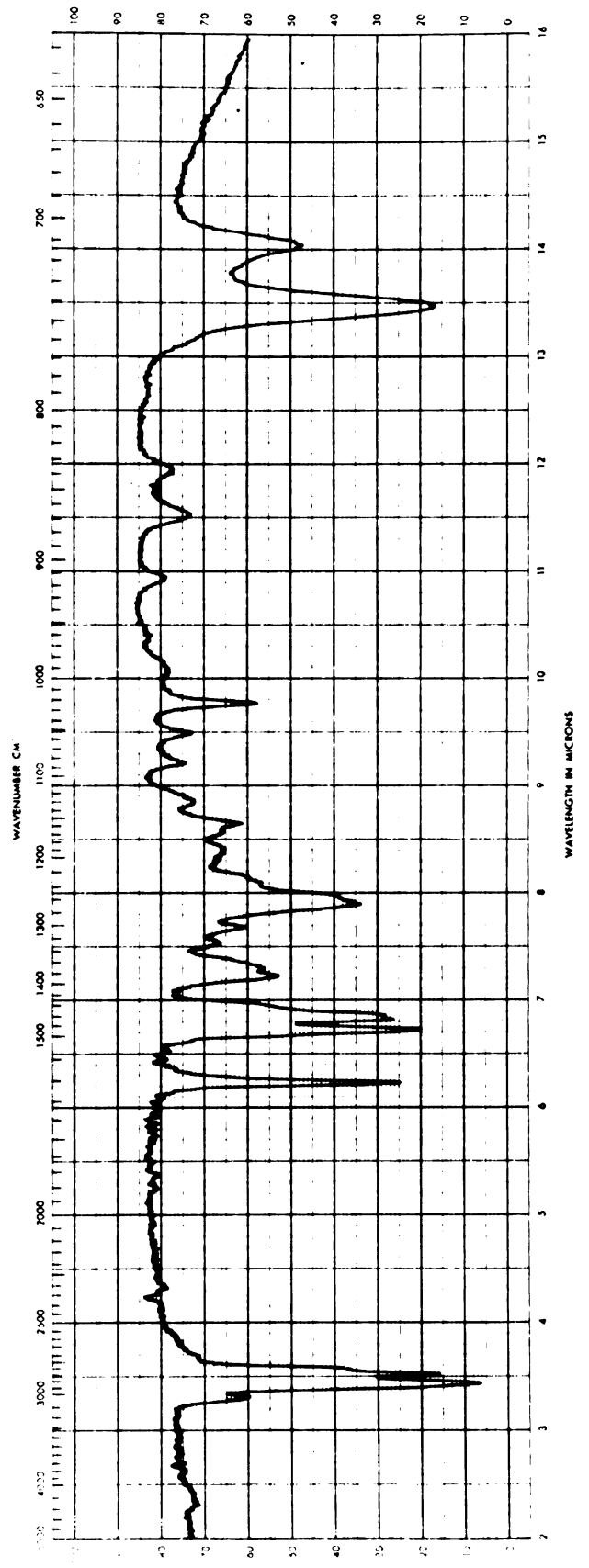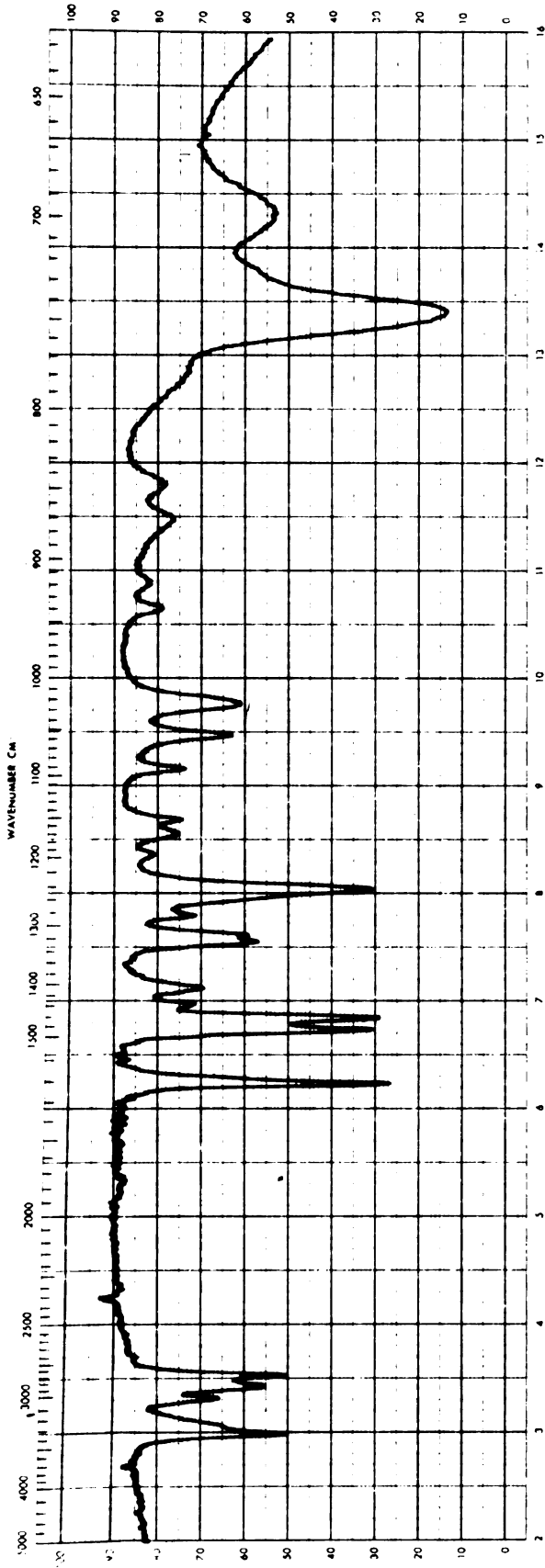
FIGURE 2

Infrared spectra for:
   Upper; Indole
   Lower; 1-n-Decylindole
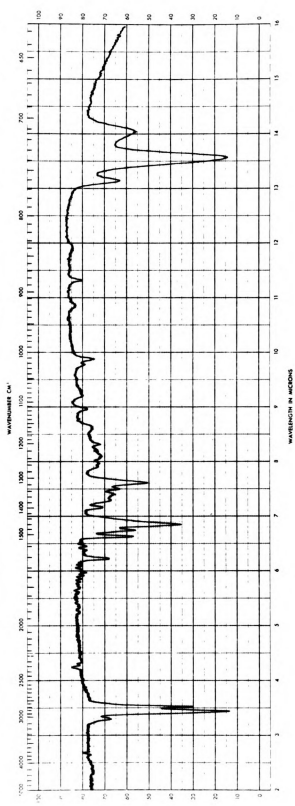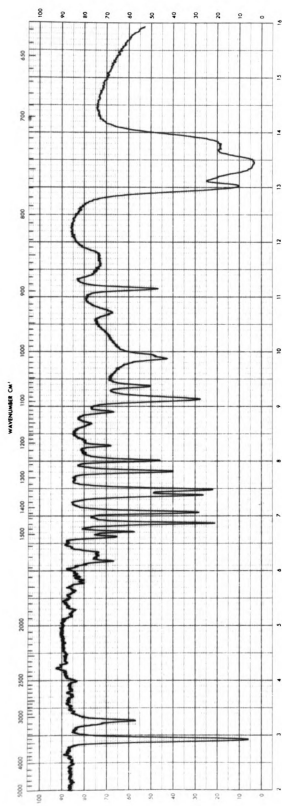
FIGURE 3

Infrared spectra for:
   Upper; Ethyl indole-3-acetate
   Lower; Ethyl 1-n-decylindole-3-
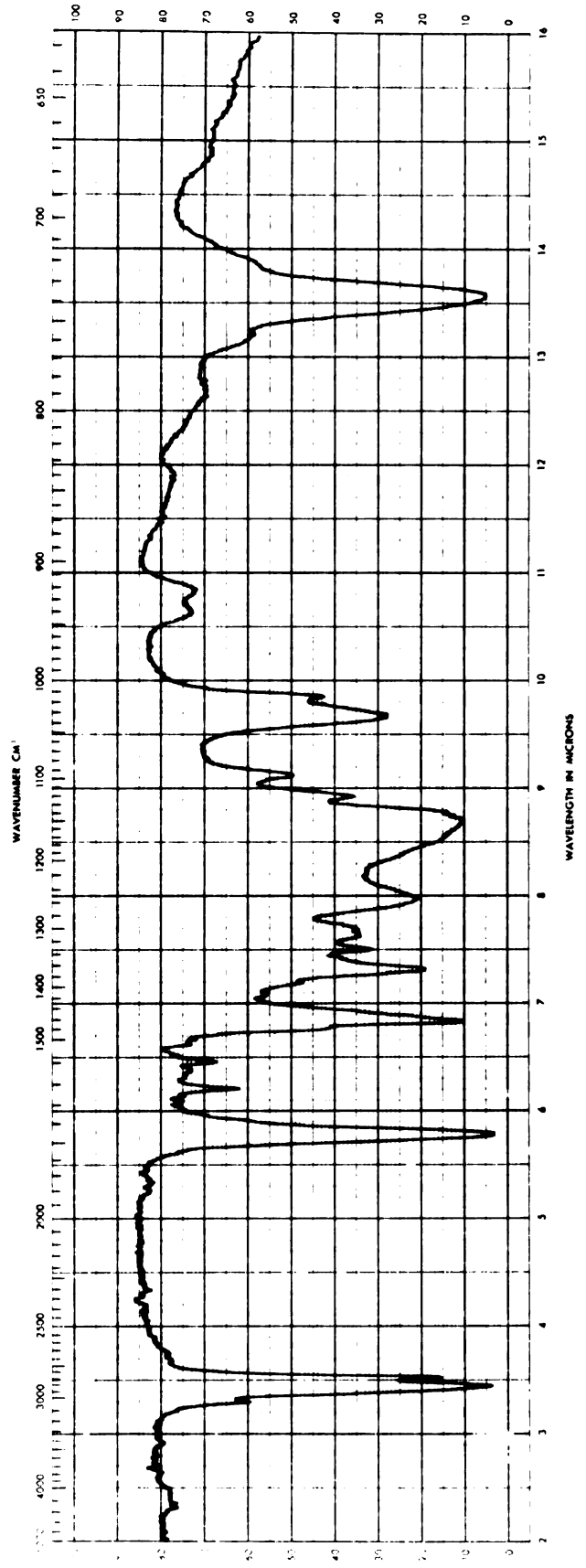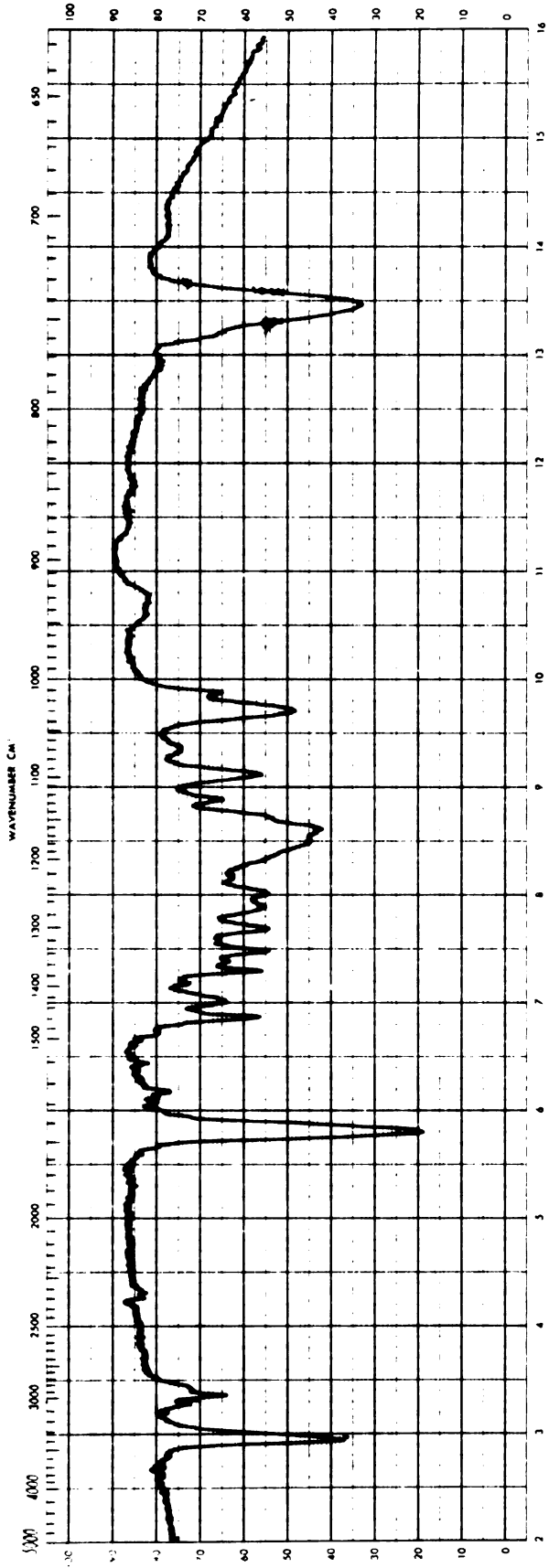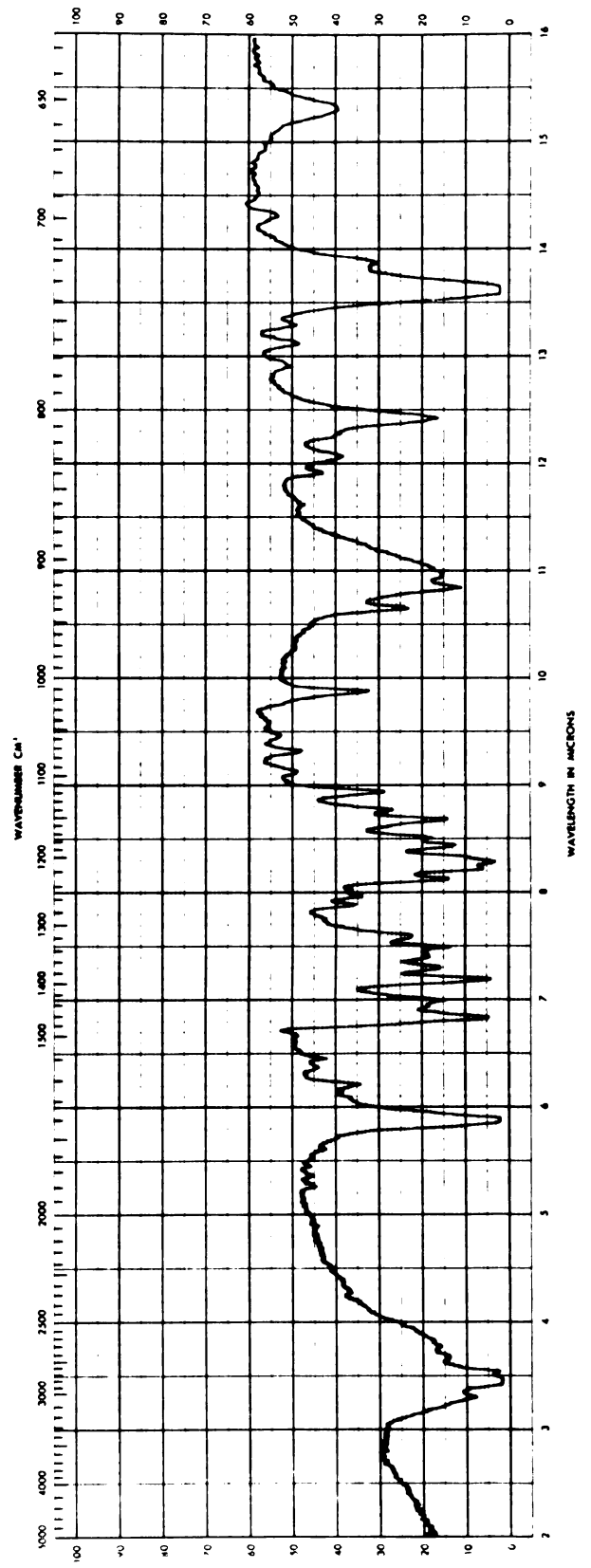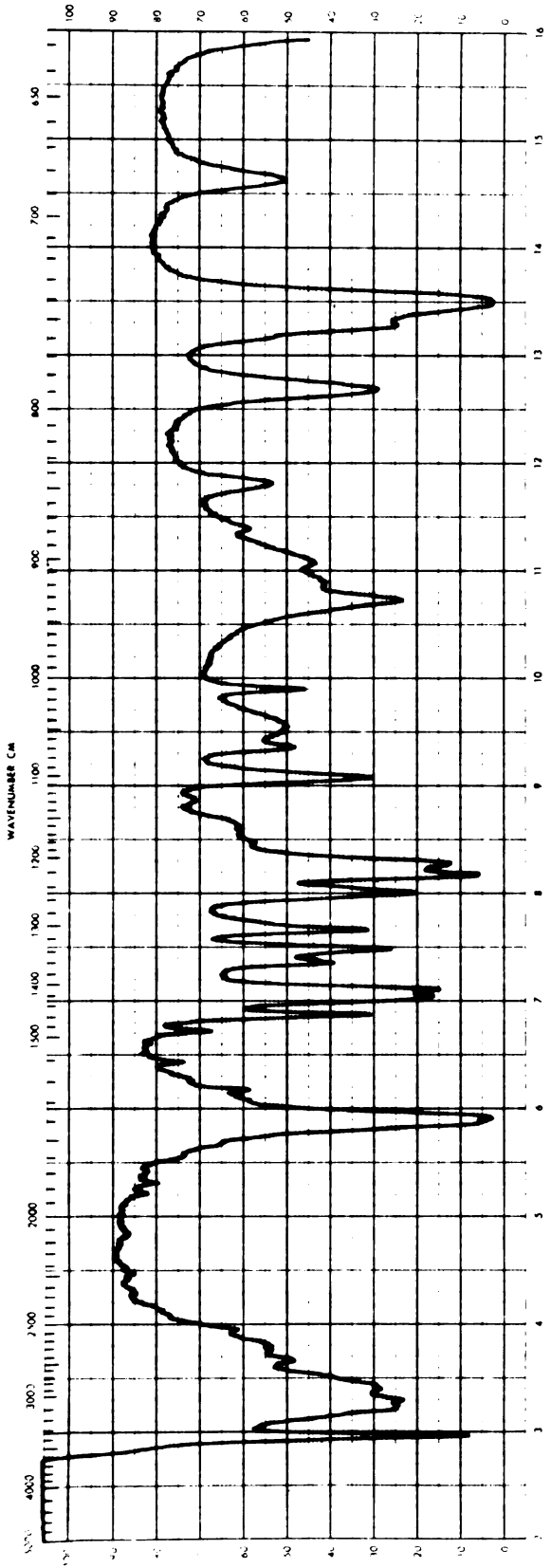   acetate

etate
dole-j-

FIGURE 4

Infrared spectra for:
   Upper; Indole-3-acetic acid
   Lower; 1-n-Decylindole-3-
        acetic acid

the 8.26 to 8.62 μ range (110). Katritzky et al. (43, 44)
report that a peak near 8.4 μ is to be expected with esters
having chains two or more atoms in length attached to each
side of the ester grouping (ROC=O).

The typical broad absorption band in the range of
3-4 μ is characteristic of the carboxylic acids (68) and
serves as definite evidence for the structure assigned in
Table 4 and Figure 4. Carbon-hydrogen stretching vibra-
tions are buried beneath the broad carboxy absorbance band
and contribute to the high frequency absorbance. An acid
dimer band near 10.5-11 μ was seen in each spectrum of the
acids. This absorption is attributable to the OH out of
plane deformation (24). The strong ester band near 8.6 μ
either disappeared or significantly changed shape upon
saponification.

Infrared absorbance near 2.9 μ was the first indica-
tion of an impure product obtained by benzylation of the
nitrogen of ethyl indole-3-acetate. Silica gel thin-layer
chromatography with either Eastman sheet 6060 or Brinkman
precoated glass plates in 18% butanone-hexane was incon-
clusive because the R_f values of the reactant ester and
that of the by-product p-substituted benzylindole-3-acetate
were indistinguishable. Hence, the thin-layer chromato-
graphic method of monitoring the reaction indicated the
presence of only the starting material (ethyl indole-3-
acetate) and the expected N-substituted indole analog.
However, the melting points and elemental analyses of the

products revealed that the reaction mixture contained the N-substituted indole compound and the corresponding benzyl ester of indole-3-acetic acid.

A bathochromic shift in the ultraviolet spectra of 1-substituted indoline and indole derivatives was very useful in characterization of reaction products (Tables 1, 2, 3, 4). The latter phenomenon was utilized in the isolation and characterization of the by-product p-substituted benzyl esters of indole-3-acetic acid. Although no extensive work has been done on the ultraviolet spectra of indole derivatives, several reports of a similar bathochromic shift are quoted in the literature. For example, Yamada et al. (126) observed this shift on alkylation of tryptophan. They reported that the peak near 290 μ changes to a shoulder which is shifted toward longer wavelengths by approximately 5 to 6 μ. Hinman and Lang (65) reported a similar value for 1-methylindole.

Substitution in the 3-position could also be verified by ultraviolet data. This is evident by considering the absorption maxima of indole-3-acetic acid relative to indole (Figures 2 and 4). In this case, the bathochromic shift is only about 2-3 μ. An additive effect is seen when a 1, 2-disubstituted indole is used. Thus, 1-alkyl indole-3-acetic acids or esters of the acids are shifted about 9 μ relative to indole and only about 3 μ relative to a 1-substituted indole.

Many of the products and intermediates in the synthetic pathways were purified for analysis on Brinkman fluores-

cent 2 mm silica gel preparative layer chromatography plates.
One hundred to 150 mg of a compound or a reaction mixture
could be separated at one time. The absorbent containing
each product was then scraped from the plate and eluted
with methanol. Liquid samples isolated in this manner were
distilled in a side-arm test tube attached to a cold finger
condenser. Solid compounds were recrystallized from
methanol-water or from benzene-hexane.

Synthesis

Indoline is a more nucleophilic compound than is
indole. This characteristic can be attributed to the
aromaticity of the pyrrole ring of indole molecule which
makes the lone pair of electrons less available for reac-
tion. The reactivity of indoline is similar to that of
aniline. In agreement with this fact was the finding that
indoline reacts with a _tert_-butyl halide to approximately
the same extent as does N-methylaniline.

In the present work, indoline was chosen as a start-
ing material for some of the 1-substituted indole-3-acetic
acids, since it was possible to make derivatives containing
bulky _iso_-propyl or _tert_-butyl moieties. The literature
reports indicate that direct alkylation of the indole
nucleus with large halide moieties has not been successful
even when the N-sodium salt was used (126). Purification
of the substituted indoline before conversion to the substi-
tuted indole was easily accomplished by using benzenesul-
fonyl chloride. Acid extraction of the product from the

amide was then possible.

A short path "Kontes" still equipped with a fraction-
ating receiver was used for distillations because it had
very little holdup. The solution in the distilling flask
was stirred vigorously with a magnetic stirring bar while
it was heated in a sand filled electric heating mantle.
This simple still was adequate for separating indoline from
the alkylated product when the chain was 5 carbons or more
in length.

Dichlorodicyanobenzoquinone was a satisfactory
dehydrogenating agent for aromatizing the substituted
indoline nucleus. However, heating in boiling xylene was
necessary for the dehydrogenation reaction to go to comple-
tion. Approximately one-half as much product was isolated
when a solution of the reactants was merely mixed and
allowed to stand at room temperature. An exothermic reac-
tion was observed when dichlorodicyanobenzoquinone was
added to a xylene solution containing indoline. Comparative
studies demonstrated that chloranil gave a reaction mixture
which was difficult to purify and afforded poorer yields
than did dichlorodicyanobenzoquinone. Neither the use of
excess chloranil nor refluxing mesitylene (bp 165°) as the
solvent proved as effective for aromatization.

Jansen et al. (36) found Attenburrow manganese
dioxide the best reagent tried for the dehydrogenation of a
tricyclic indoline. Their yield of 64% was approximately
the same as those reported herein. Russian investigators
(97) employed chloranil for the aromatization of a glucosyl

indoline, but later utilized dichlorodicyanobenzoquinone
for a similar reaction (78). In the present study, the
reaction worked as well for p-substituted benzylindolines
as it did for the alkylindolines.

Snyder and Eliel (90) and Katritzky (42) indicated
that whereas 1-methylindole underwent a normal Mannich
reaction and formed the expected methiodide salt, the
alkylation with cyanide proceeded in an anomalous manner.
In addition to the dimethylamino substitution product,
1-methylindole-3-acetonitrile, a significant amount of
isomeric by-product was formed. Snyder and Eliel (90)
demonstrated this phenomenon to be an allylic rearrangement
to produce 2-cyano-1,3-dimethylindole.

As a representative compound, 1-n-decylindole was
chosen for the investigation of this synthetic route. The
side-chain was successfully attached to the indole ring by
the Mannich reaction employing formaldehyde and dimethyl-
amine (51). The structure of the reaction product was
verified by infrared and ultraviolet spectra. 1-n-Decyl-
gramine had the typical bathochromically displaced shoulder
at 297 mμ. In the infrared region, the presence of a
dimethylamine moiety was indicated by a doublet at 3.58
and 3.64 μ (12). Although the distilled product had no
N-H stretch, it did have the intense aliphatic C-H bands
characteristic of the n-decyl chain.

Stowe (93) indicated a preference of the methosulfate
of gramine over the methiodide as an intermediate in the

alkylation of gramine. In deference to Stowe's study, the methosulfate of 1-n-decylgramine was reacted with excess dimethyl sulfate and a purified tetrahydrofuran solution of the alkyl gramine. White crystals of the salt formed after the solution stood overnight in the cold. The product was characterized only by its melting point which was 99-101°.

To determine whether two compounds were produced in the alkylation step, the methosulfate salt was reacted with a 3-fold excess of potassium cyanide by refluxing in a dimethylformamide solution according to a method outlined by Hill (32). After extraction with ethyl ether and removal of the solvent, the product was purified by distillation and the infrared spectrum examined. The most striking features of the spectrum were the absence of a N-dimethyl doublet and the presence of a nitrile band at 4.35 μ. The latter material was purified by preparative layer chromatography. Two distinct bands were observed which were separately eluted and examined with ultraviolet spectrometry. One band contained material which had the expected spectrum of 1-n-decylindole-3-acetonitrile whereas that from the other band showed a series of peaks at longer wavelengths, which are not typical of indole compounds. The latter band contained a compound which was assumed to be the 2-nitrile homolog of the material isolated by Snyder and Eliel (90).

Katritzkǂ (42) found that a potassium hydroxide solution perferentially hydrolyzed 1-methylindole-3-acetonitrile

to the corresponding acid when the isomeric mixture was saponified. However, with the mixture investigated herein the base extractable material from the saponification melted at a lower temperature than did the 1-n-decylindole-3-acetic acid synthesized by employing ethyl diazoacetate. Therefore, the synthetic sequence for attachment of the side chain involving the Mannich base was abandoned in favor of ethyl diazoacetate.

Nametkin et al. (69) investigated the effect of various conditions on the reaction of ethyl diazoacetate with indole. They concluded that cuprous chloride was the most effective catalyst and on the basis of their finding the same catalyst was used in the present work.

Several Merck investigators (60 to 65) have synthesized a large number of 1-benzylated indole derivatives. Among these are several compounds closely related to 1-p-benzylindole-3-acetic acid. In their synthetic scheme these investigators used the N-sodium salt of the indole compound in question. Dimethylformamide, a highly polar aprotic solvent which solvated the newly formed salt, was used for the reaction medium. Reaction of the N-sodium salt with a benzyl halide yielded the 1-benzyl derivative of the indole. They made no reference to any by-products. This procedure was followed in the preparation of 1-p-substituted benzyl derivatives of ethyl indole-3-acetate. Significantly, a considerable quantity of by-product was also obtained by this method. The by-product was inseparable

from the desired compound by simple vacuum distillation.
Fractional crystallization from methanol-water of either
the reaction mixture or the distillate afforded partial
purification of the product. To obtain chromatographi-
cally pure material, preparative layer chromatography was
utilized and the by-product was separated from the main
product. Elemental and spectral analysis revealed that
the by-product was the corresponding p-substituted benzyl
indole-3-acetate (Figure 6).

The structure of ethyl p-chloroindole-3-acetate
was verified by synthesizing it by another route. Indoline
was reacted with p-chlorobenzyl chloride and the resultant
product (Figure 5) was dehydrogenated. Reaction of the
substituted indole with ethyl diazoacetate yielded the
same material as that made from ethyl indole-3-acetate.
Superimposition of the infrared spectra was possible as
can be seen in Figure 6. Tables 5 through 8 list the
physical data of the benzyl derivatives and the intermedi-
ates used in the syntheses.

The synthesis of methyl-1-glucosylindole-3-acetate
involved the same general route as outlined for the 1-alkyl
indole-3-acetic acids. Several attempts to repeat the
synthesis of 1-(2',3',4',6'-tetra-O-acetyl-β-D-glucopyran-
osyl)indole by the procedure of Suvorov and Preobrazhenskoyz
(97, 98) were unsuccessful. Although reasonable yields of
the intermediate 1-glucosyl indoline were obtained, diffi-
culty was encountered in dehydrogenation of the indoline

FIGURE 5

Infrared spectra for:
   Upper; p-Chlorobenzylindoline
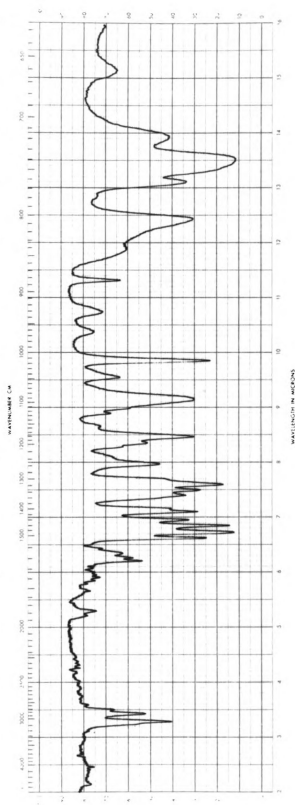   Lower; p-Chlorobenzylindole
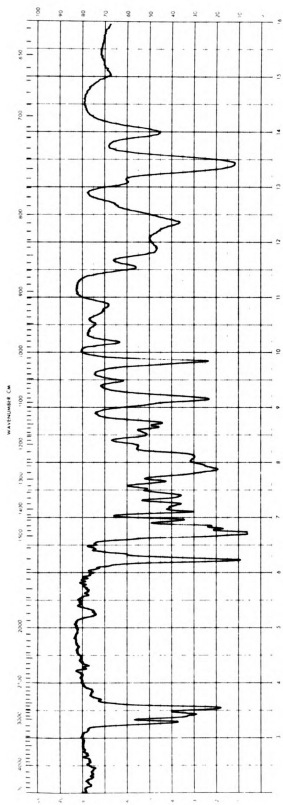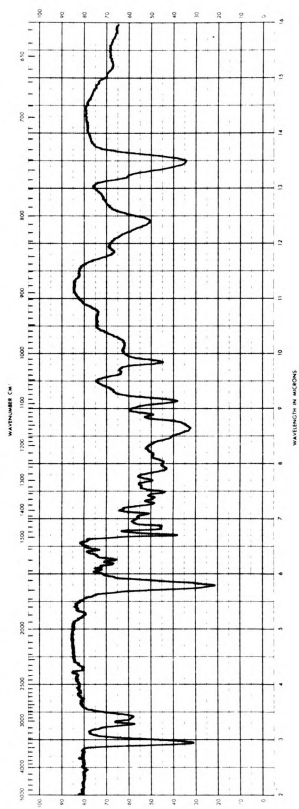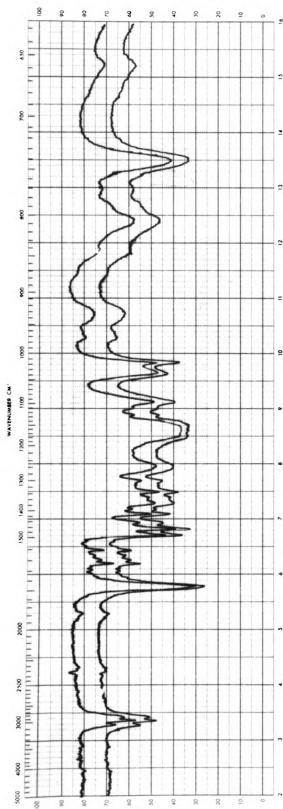
FIGURE 6

Infrared spectra for:
    Upper; Ethyl 1-p-chlorobenzylindole-
            3-acetate
            Top, Synthesized from indoline
            Bottom, Synthesized from ethyl
                indole-3-acetate
    Lower; p-Chlorobenzylindole-3-acetate

Table 5. Physical Data for Ethyl-1-_para_-Substituted Benzylindole-3-Acetates

Compound	Molecular Weight	Melting Point[1] Degrees C	Absorption Maxima[3]			Calcd. % N	Found % N
			uv[2] mμ	C=O μ	R-C=O μ		
Ethyl 1-benzyl-indole-3-acetate	293.37	44-44.5	277s 285 296	5.78	8.70[4]	4.77	4.77
Ethyl 1-p-fluoro-benzylindole-3-acetate	311.36	69	267 272 285 295	5.79	8.64	4.50	4.47
Ethyl 1-p-chloro-benzylindole-3-acetate	327.81	96-98	277 285 295	5.79	8.60	4.27	4.22
Ethyl 1-p-bromo-benzylindole-3-acetate	372.27	104-104.5	274 283 296s	5.79	8.45	3.76	3.81

Compound	Mol. wt.	M.p.	UV	IR		
Ethyl 1-p-methyl-benzylindole-3-acetate	307.39	65.5	275 285 296s	5.79 8.70	4.56	4.61
Ethyl 1-p-methoxy-benzylindole-3-acetate	323.39	38.5–40	278 284 297s	5.79 8.60	4.33	4.25
Ethyl 1-p-nitro-benzylindole-3-acetate	338.36	101	273 295	5.79 8.47	8.28	8.01

[1] Melting points were made on a Fisher-Johns apparatus and are uncorrected.

[2] Ultraviolet spectra were determined in 95% ethanol solutions.

[3] Infrared spectra were measured on potassium bromide pellets.

[4] An obvious peak was also seen at 14.3 (5 adj. hydrogen).

Table 6. Physical Data for 1-para-Substituted Benzylindole-3-Acetic Acids

Compound	Molecular Weight	Melting Point[1] Degrees C	Absorption Maxima[3]				
			uv[2] mµ	O-H µ	C=O µ	Calcd.	Found
1-Benzylindole-3-acetic acid	265.31	155-156	277s 286 296s	3-4	5.88	5.28	---
1-p-Fluorobenzyl-indole-3-acetic acid	283.30	159.5-160.5	267 273 289 296s	3-4	5.86	4.94	4.96
1-p-Chlorobenzyl-indole-3-acetic acid	299.76	145.5-147.5	277 285 295s	3-4	5.88	4.67	4.68
1-p-Bromobenzyl-indole-3-acetic acid	344.21	152	277 285 295	3-4	5.87	4.07	4.06

			274 286 296s				
1-p-Methylbenzyl-indole-3-acetic acid	279.34	125-126		3-4	5.86	5.01	5.00
1-p-Methoxybenzyl-indole-3-acetic acid	295.34	136-137	278 284 296s	3-4	5.87	4.74	4.85

[1]Melting points were made on a Fisher-Johns apparatus and are uncorrected.

[2]Ultraviolet spectra were determined in 95% ethanol solutions.

[3]Infrared spectra were measured on potassium bromide pellets.

Table 7. Physical Data for Other Indole and Indoline Intermediates

Compound	Molecular Weight	Melting Point[1] Degrees C	Absorption Maxima[3]				Calcd. % N	Found % N
			uv[2] mμ	OH μ	C-H μ	C=O μ		
1-(2',3',4',6'-Tetra-O-acetyl-β-D-glucopyranosyl) indoline	449.44	117-118	246 295	---	3.52	5.71	3.12	---
1-(2',3',4',6'-Tetra-O-acetyl-β-D-glucopyranosyl) indole	447.43	148-149.5	263 277 289	---	3.48	5.71	3.13	---
Ethyl 1-(2',3',4',6'-Tetra-O-acetyl-β-D-glucopyranosyl) indole-3-acetate	533.53	138-139	267 279 290	---	3.37	5.74	2.625	2.87
Methyl 1-β-D-glucopyranosyl-indole-3-acetate	351.36	163-164	272 279 290	2.75-3.2	3.43	5.83	3.99	3.96

1-p-Chlorobenzyl-indoline	243.73	26-27	253 295	---	3.3	---	5.75 5.99
1-p-Chlorobenzyl-indole	241.71	---	270 292	---	3.3	---	5.80 5.66
1-p-Nitrobenzyl-indoline	254.30	98.5-99.5	258	---	3.29	---	11.02 10.95
1-p-Nitrobenzyl-indole	252.28	102	269 292	---	3.29	---	11.11 11.10

[1] Melting points were determined on a Fisher-Johns apparatus and are uncorrected.

[2] Ultraviolet spectra were made in 95% ethanol solutions.

[3] See text for details of infrared spectra.

Table 8. Physical Data for para-Substituted Benzylindole-3-Acetates

Compound	Molecular Weight	Melting Point[1] Degrees C	Absorption Maxima[3]				Calcd. % N	Found % N
			uv[2] mµ	N-H µ	C=O µ	ROC=O µ		
p-Methylbenzyl indole-3-acetate	279.34	70-71	273 280 290	2.99	5.81	8.55	5.01	4.87
p-Chlorobenzyl indole-3-acetate	299.76	95.5	279 290	2.94	5.79	8.65	4.67	4.67
p-Bromobenzyl indole-3-acetate	344.21	102.5-104	273 280 290	2.94	5.79	8.64	4.07	4.28
p-Nitrobenzyl indole-3-acetate	310.31	114	270 289	2.98	5.79	8.56	9.03	8.87

[1]Melting points were determined on a Fisher-Johns apparatus and are uncorrected.

[2]Ultraviolet spectra were determined in 95% ethanol solutions.

[3]Infrared spectra were measured on potassium bromide pellets.

ring with chloranil. The melting point of the isolated reaction product was variable and the ultraviolet spectrum indicated a mixture of an indoline and an indole. Dichloro-dicyanobenzoquinone, however, proved completely satisfactory for this and all other aromatizations investigated. The by-product, a substituted hydroquinone, was simply filtered from the cooled xylene solution. Care must be exercised so that the solution is not cooled too much because the indole-sugar derivative will crystallize from cold xylene. Figure 7 shows the infrared spectra of the intermediates and the product of this synthetic scheme.

Attachment of the side-chain to the glucosyl-indole went smoothly only if xylene was used as the solvent in preference to benzene. The ethyl ester prepared from ethyl diazoacetate was purified by column chromatography and crystallized from methanol-water. The acetate blocking groups were effectively removed by treating a cold methanol solution of the ester with barium methoxide as described for similar compounds by Isbell (34). Deacetylation was attended by transesterification of the side-chain to yield the deacylated methyl ester. Attempts to hydrolyze the methyl ester resulted in a product that had a lower R_f value on thin-layer chromatography than did the ester. The hydrolytic product did not crystallize.

The spectra of Figure 7 serve to verify that each reaction step took place. The center spectrum has a wider carbonyl band than the starting material (upper spectrum)
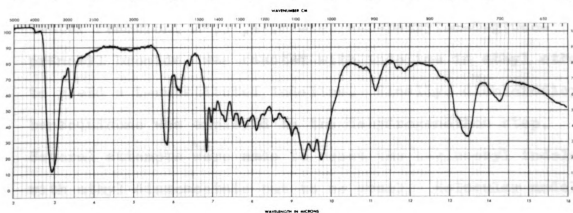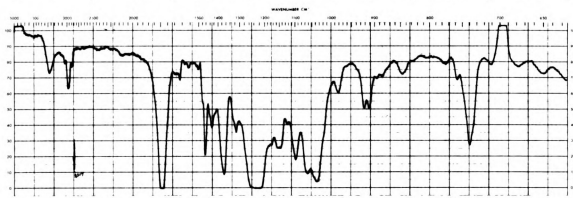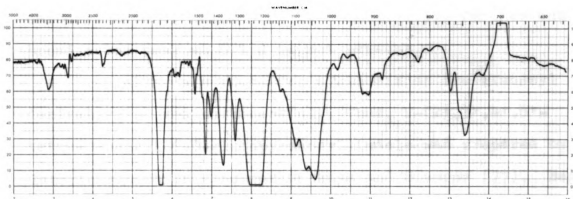
FIGURE 7

Infrared spectra of glucosyl derivatives:
    Upper; 1-(2',3',4',6'-Tetra-O-acetyl-β-D-
        glucopyranosyl)indole
    Center; Ethyl 1-(2',3',4',6'-tetra-O-acetyl-
        glucopyranosyl)indole-3-acetate
    Lower; Methyl 1-β-D-glucopyranosyl-
        indole-3-acetate

which was shifted to a slightly longer wavelength. Increased C-H absorption is also noted and a new ester band appears at 8.7 μ. A large hydroxyl band is evident in the deacetylated product as shown in the lower spectrum. In this case the carbonyl band at about 5.85 is shifted still farther and the broad ester peak at 8.1 μ is not present.

An alternate route for synthesis of the glucosyl derivative proved unsuccessful. Thus, 1-(2',3',4',6'-tetra-O-benzyl-β-D-glucopyranosyl)indole was prepared by the laborious route of Preobrazhenskaya and Suvorov (77). Beginning with benzylation of methyl-α-D-glucoside and treatment of the product with hydrochloric acid in acetic acid one obtains a glucose derivative blocked in all but the 1-position with benzyl groups (84). A methylene chloride-pyridine solution of this material was treated with p-nitrobenzoyl chloride and the ester product was treated in turn with hydrobromic acid in methylene chloride. The product of the latter reaction, 2',3',4',6'-tetra-O-benzyl-α-D-glucopranosyl bromide, was reacted with indoline in ethyl ether to yield a 1-substituted indoline. Finally, dichlorodicyano-benzoquinone was employed in the dehydrogenation step. Several attempts were made to react the 1-glucosyl indole with ethyl diazoacetate but to no avail. Molecular models of the compound indicate that considerable steric hinderance is encountered in an attempted reaction at the 3-position of the indole moiety.

An equally fruitless effort was made to react the N-mercury salt of indole with acetobromoglucose in analogy to

the synthesis of thymine derivatives reported by Fox et al. (20).

Biological Activity

Effect of 1-alkylation on biological activity

The effect of alkyl substitution on the nitrogen atom of indole-3-acetic acid was measured by different assays. The 1-alkyl compounds were not active enough to cause significant parthenocarpic activity in the tomato ovary assay as depicted in Figure 8. Experiments indicate that fruit-setting effectiveness decreased as the chain length and size were increased.

The time required for abscission of the treated tomato ovary is a measure of activity. These data are given in Figure 9. The indole compounds were assayed at three different concentrations--10^{-3}, 10^{-4}, and 10^{-5} $\underline{M}$. Significantly, all of the derivatives, even at 10^{-5} $\underline{M}$, showed some degree of activity in this assay. However, the relative effectiveness of these derivatives cannot be assigned on the basis of either the fruit-set or ovary abscission time assay.

Bean petiole abscission was also investigated as a means of determining the effect of alkylation at the nitrogen atom. Figure 10 gives the results of this method. Unfortunately, the relative activity could not be estimated by these data because all of the alkyl indole derivatives showed very little response.

Figure 11 demonstrates the striking decrease in

FIGURE 8

Tomato Fruit-Set Assay

Effect of 1-alkylation on tomato ovary growth at 10 days fol-
lowing treatment with 10^{-3} $\underline{M}$ lanolin solutions of the test
compounds. The control abscissed at 6 days when the diameter
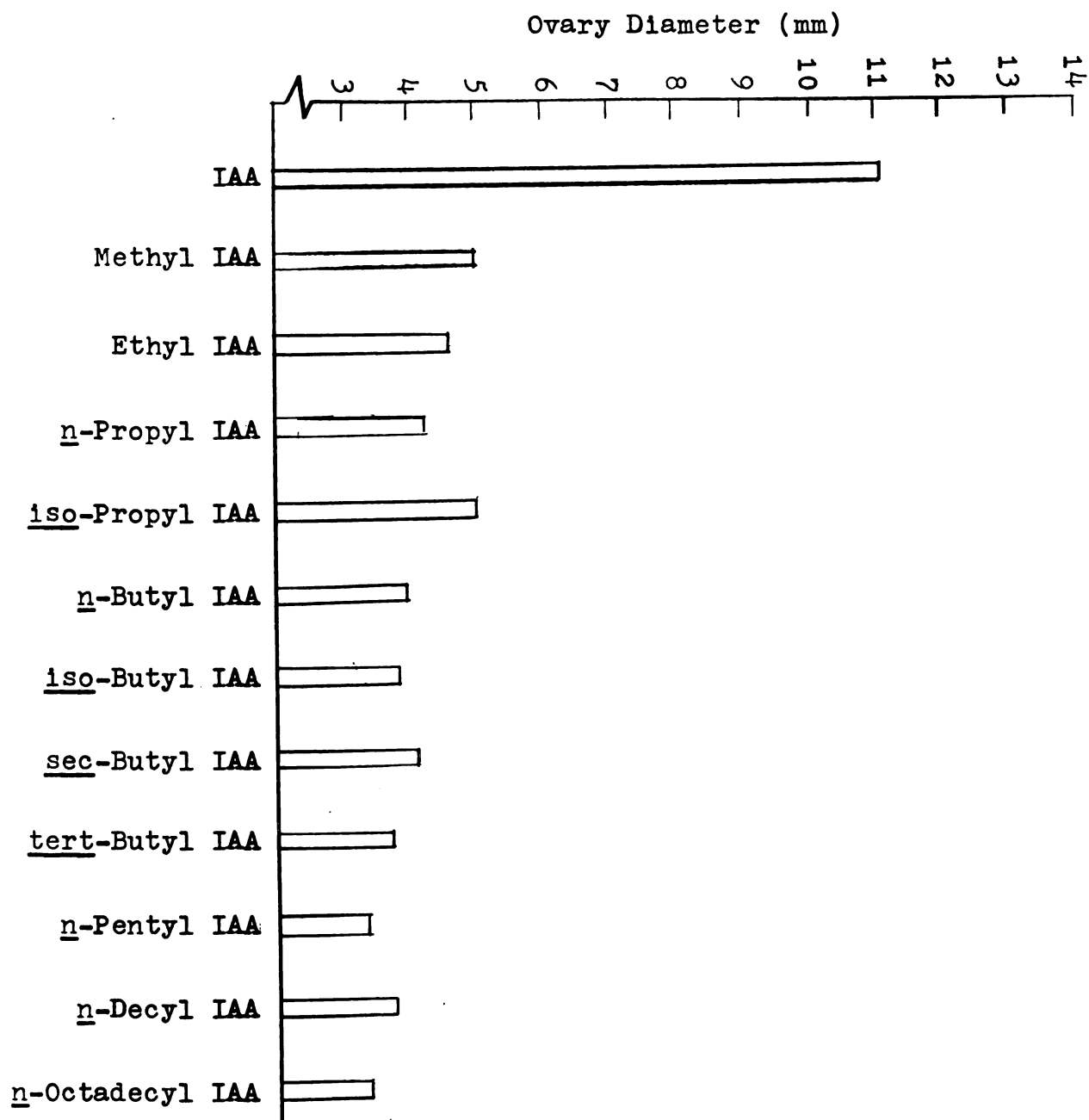was 3.5 mm.

Ovary Diameter (mm)

FIGURE 9

Tomato Ovary Abscission Assay

Abscission time of tomato fruit treated with 10^{-3} $\underline{M}$ lanolin solutions of the test compounds. Starred (*) compounds at the indicated concentrations did not abscise during the course of this experiment.

Abscission Time (Hrs)

Percent of Control
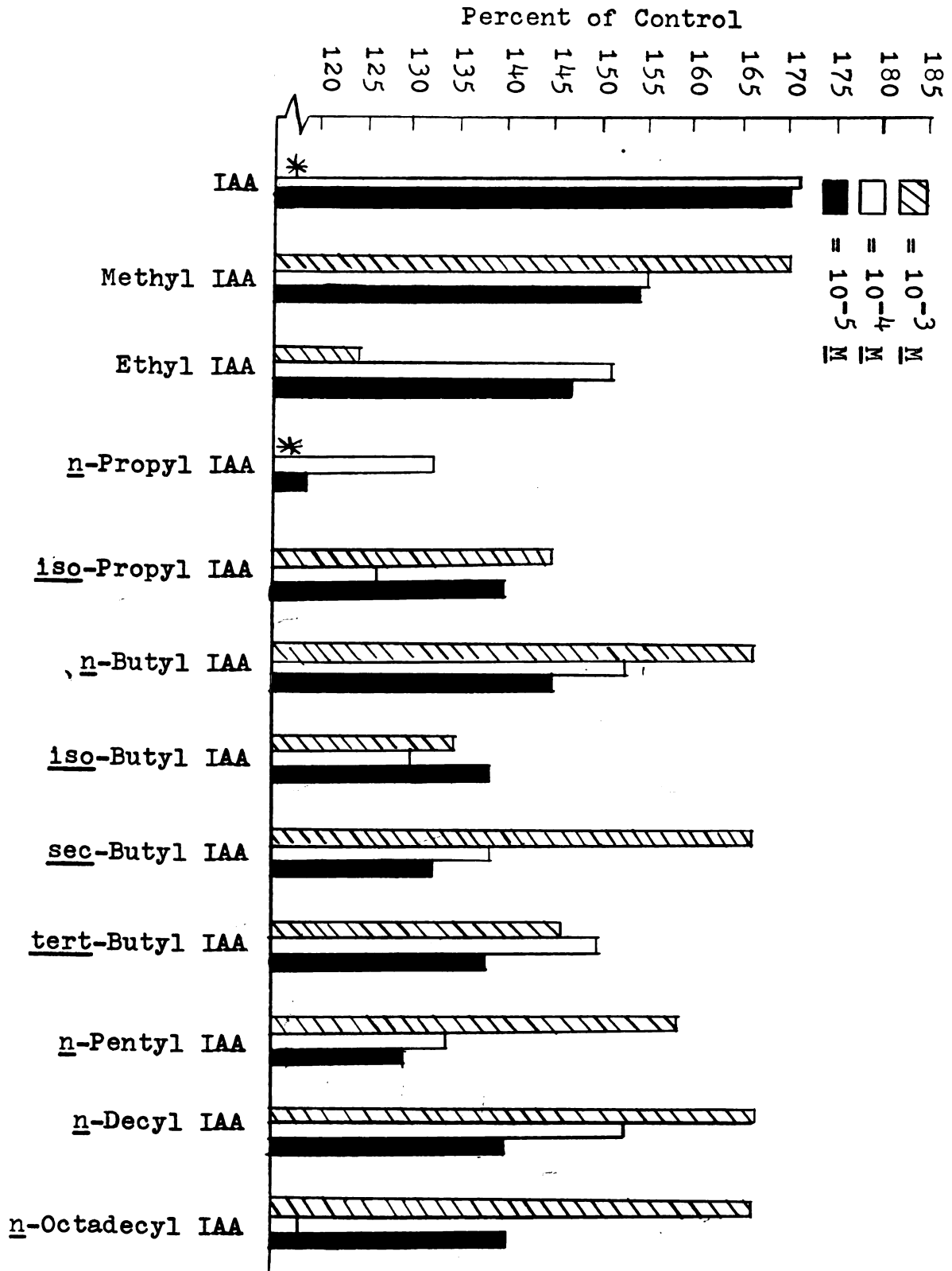
FIGURE 10

Bean Petiole Abscission Assay

Effect of 1-substitution on abscission time of debladed petiole following treatment with 10^{-3} $\underline{M}$ lanolin solutions of the test compounds.
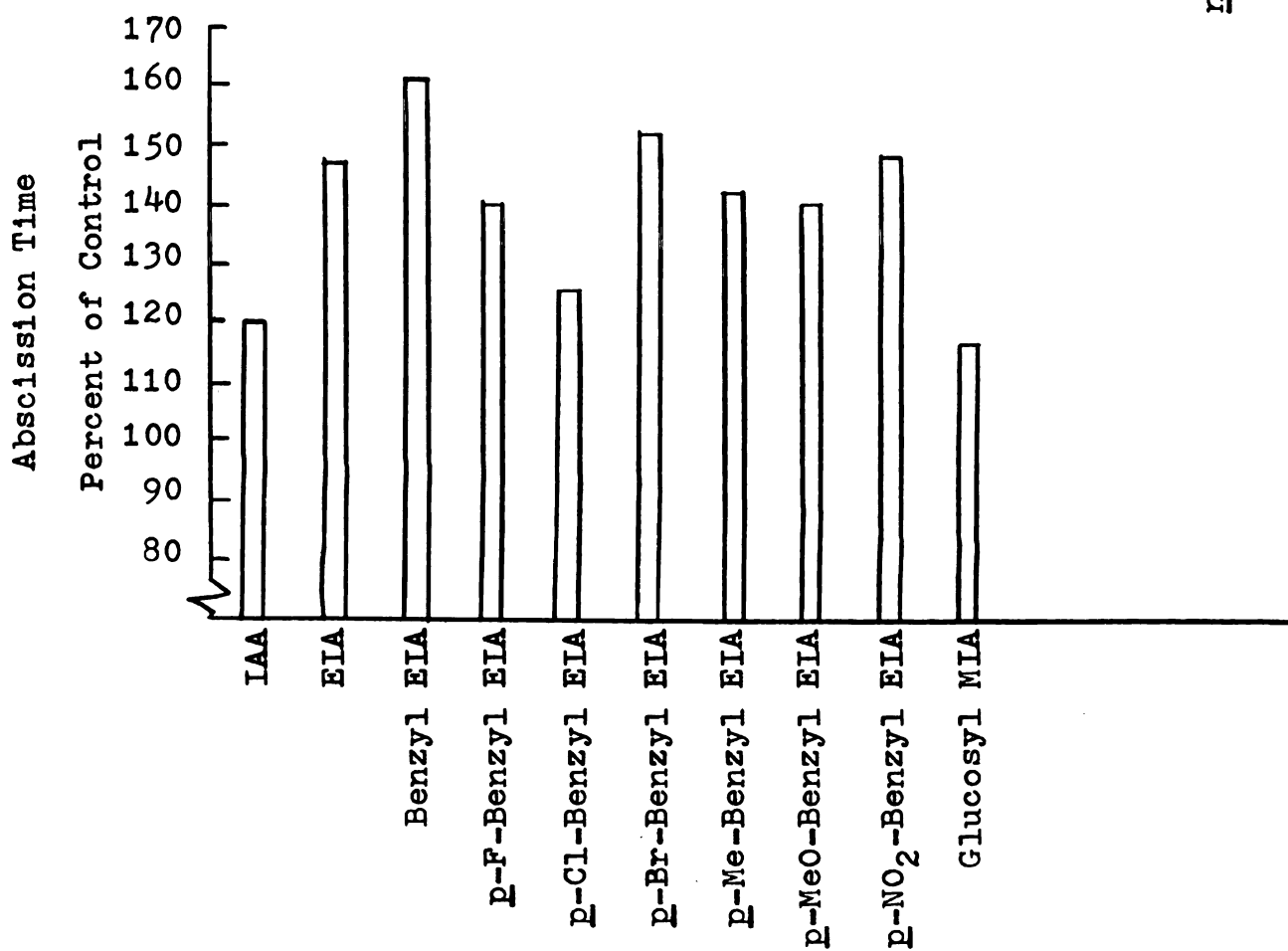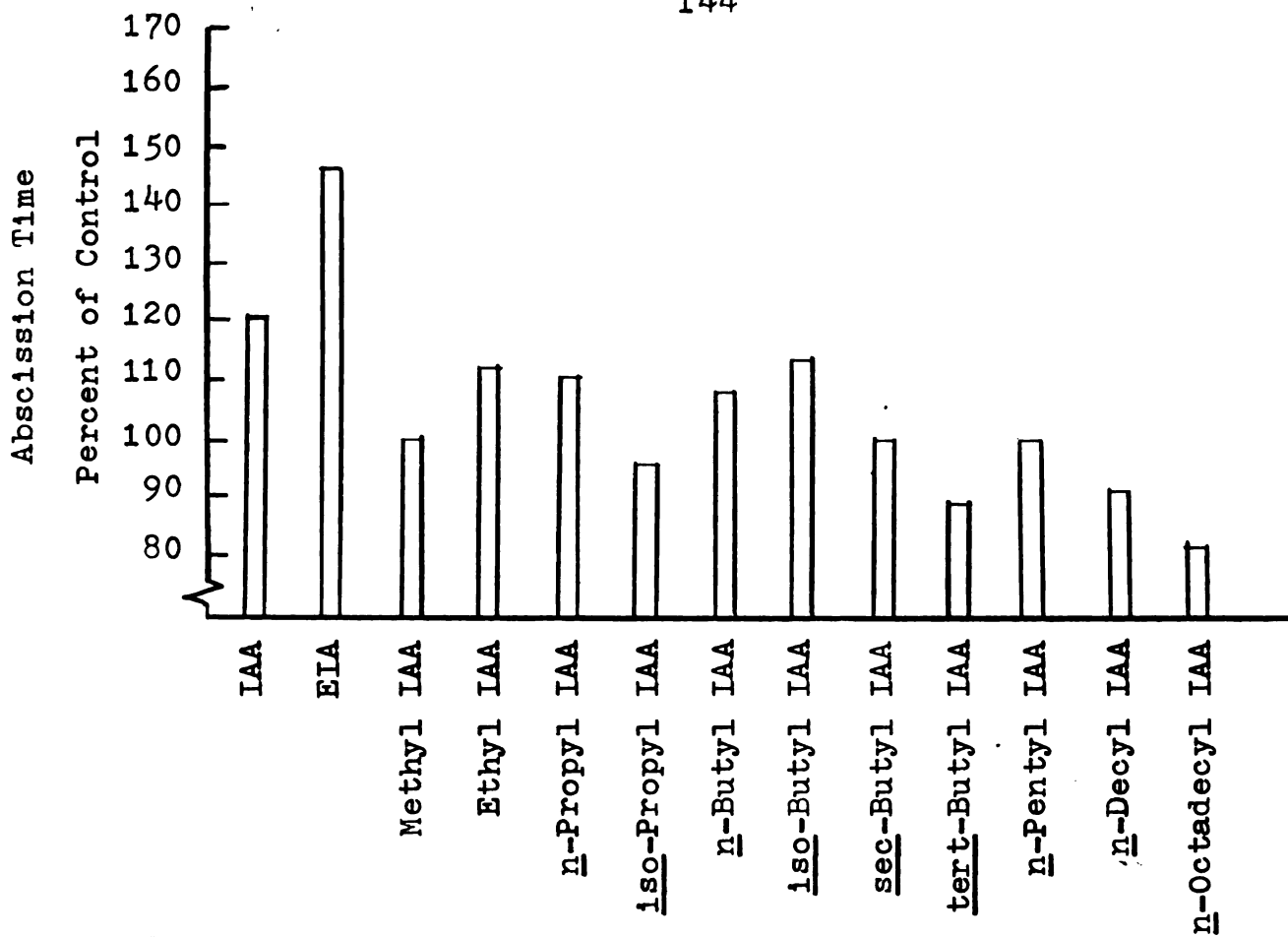
FIGURE 11

Buckwheat Root Inhibition Assay

Effect of various 1-substituted indole-3-acetic acids and esters at 10^{-4} $\underline{M}$ concentration on buckwheat root inhibition.

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Percent of IAA Inhibition at 10^{-4} $\underline{M}$
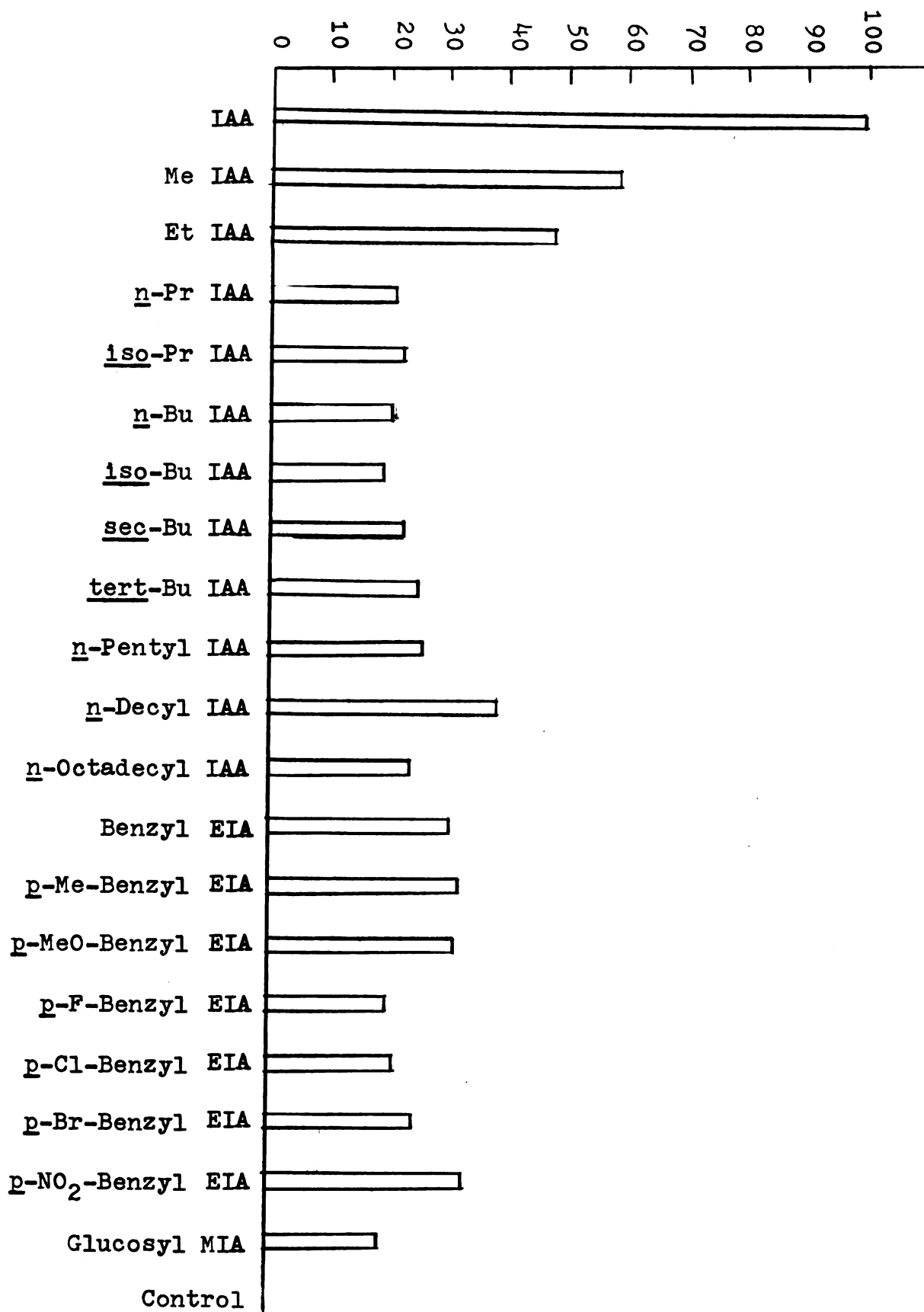
biological activity associated with increased chain length and size as determined by the buckwheat root inhibition test. The data which are presented as percent of indole-3-acetic acid inhibition, indicate a plateau in biological activity when the chain length reaches about 3 or 4 carbon atoms. Only data for the 10^{-4} $\underline{M}$ solutions are given here. More dilute solutions were less active as is evident in Figure 12.

The cucumber curvature test was employed to determine whether the alkyl indole compounds could be absorbed and translocated in an intact plant. A very unusual response was noted. Only two compounds from the 1-substituted indole acid derivatives analyzed exhibited significant activity. 1-Methylindole-3-acetic acid had a rather low degree of biological activity which appeared after about 24 hours. Much more striking was the response elicited by 1-iso-propyl-indole-3-acetic acid. The kinetics of these responses are summarized in Figures 13 and 14.

In Figure 13, curve 1 shows the time course for the effect elicited by treating one cotyledon of a cucumber seedling with 10^{-3} $\underline{M}$ indole-3-acetic acid in lanolin. The free acid causes the maximum curvature at about 2 hours followed by a slow return to the original state. In contrast, 1-iso-propylindole-3-acetate causes slow, nearly linear bending which reaches its maximum in about 30 hours and persists there for a least one week.

Treatment of one cotyledon with 1-iso-propylindole-3-acetic acid and the other with the parent acid results in

FIGURE 12

Buckwheat Root Inhibition Assay

Effect of 1-alkylation on buckwheat root inhibition: (1) IAA,
(2) Methyl IAA, (3) Ethyl IAA, (4) n-Propyl IAA, (5) iso-
Propyl IAA, (6) n-Butyl IAA, (7) sec-Butyl IAA, (8) iso-Butyl
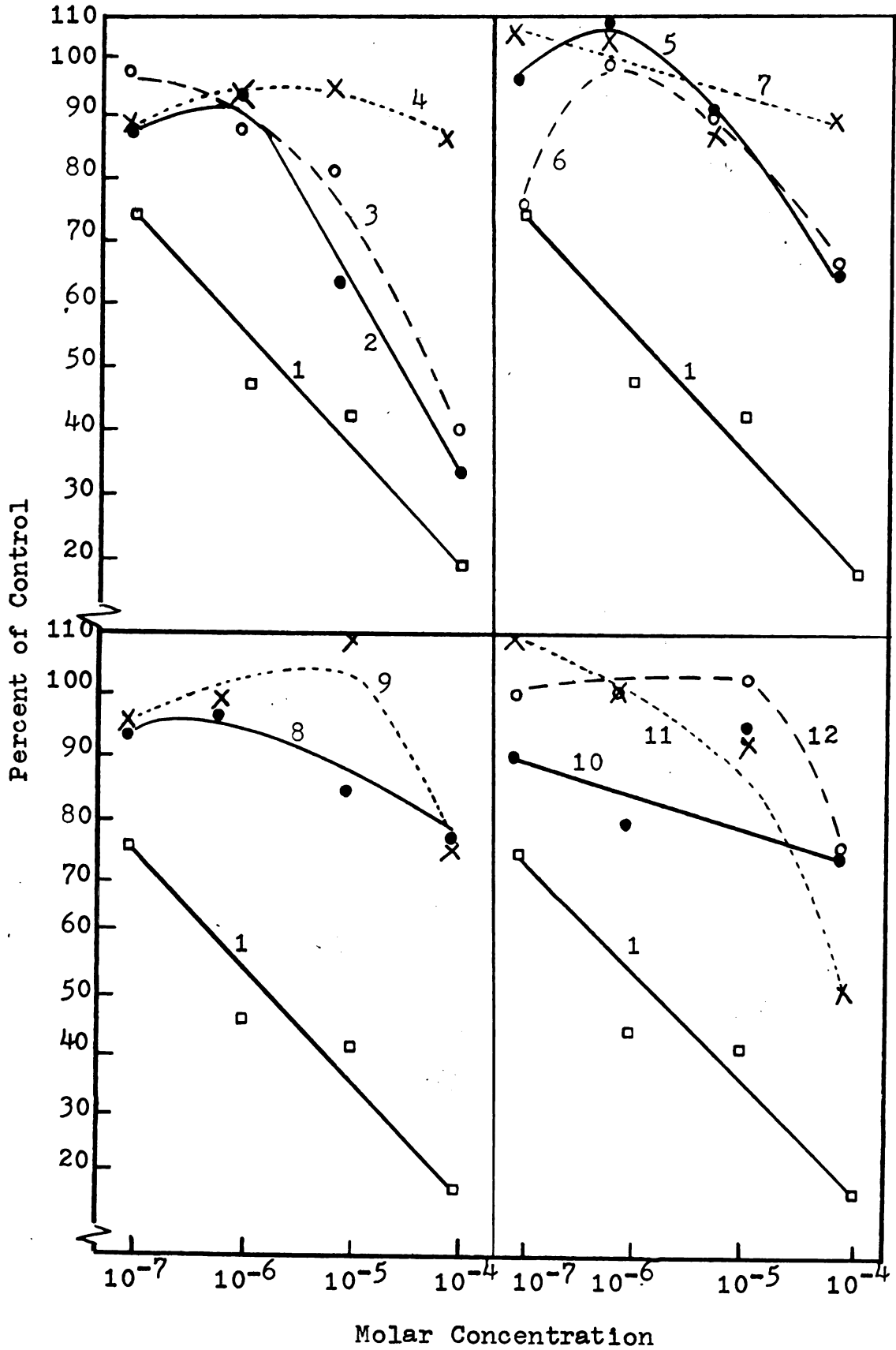IAA, (9) tert-Butyl IAA, (10) n-Pentyl IAA, (11) n-Decyl IAA,
(12) n-Octadecyl IAA.

FIGURE 13

Cucumber Curvature Assay

Kinetics of the curvature for cucumber seedlings treated with various 1-substituted indole-3-acetic acids: (1) IAA, (2) iso-Pr IAA, (3) Me IAA, (4) All of the alkyl and benzyl derivatives of indole-3-acetic acids assayed except (2) and (3).
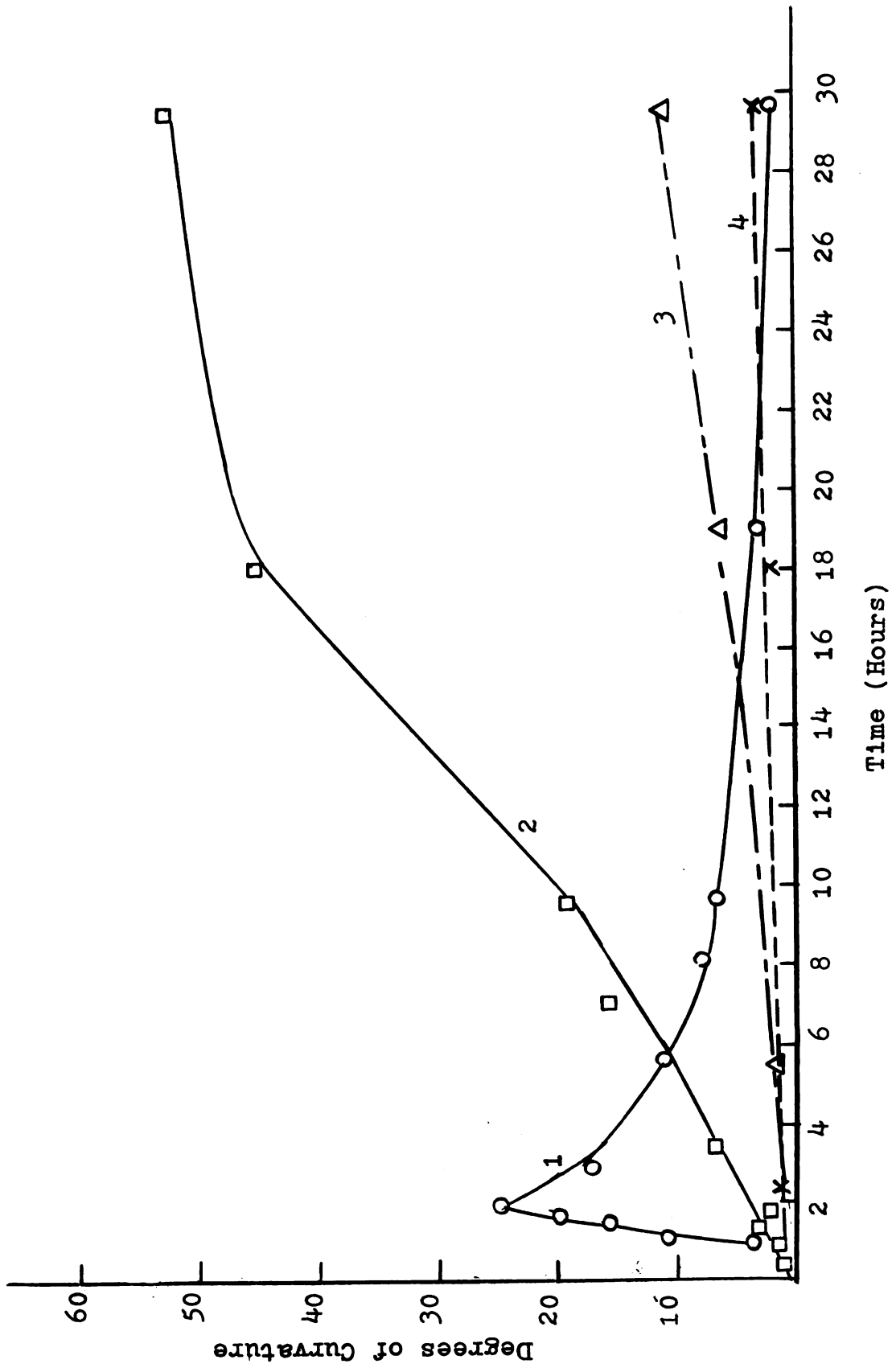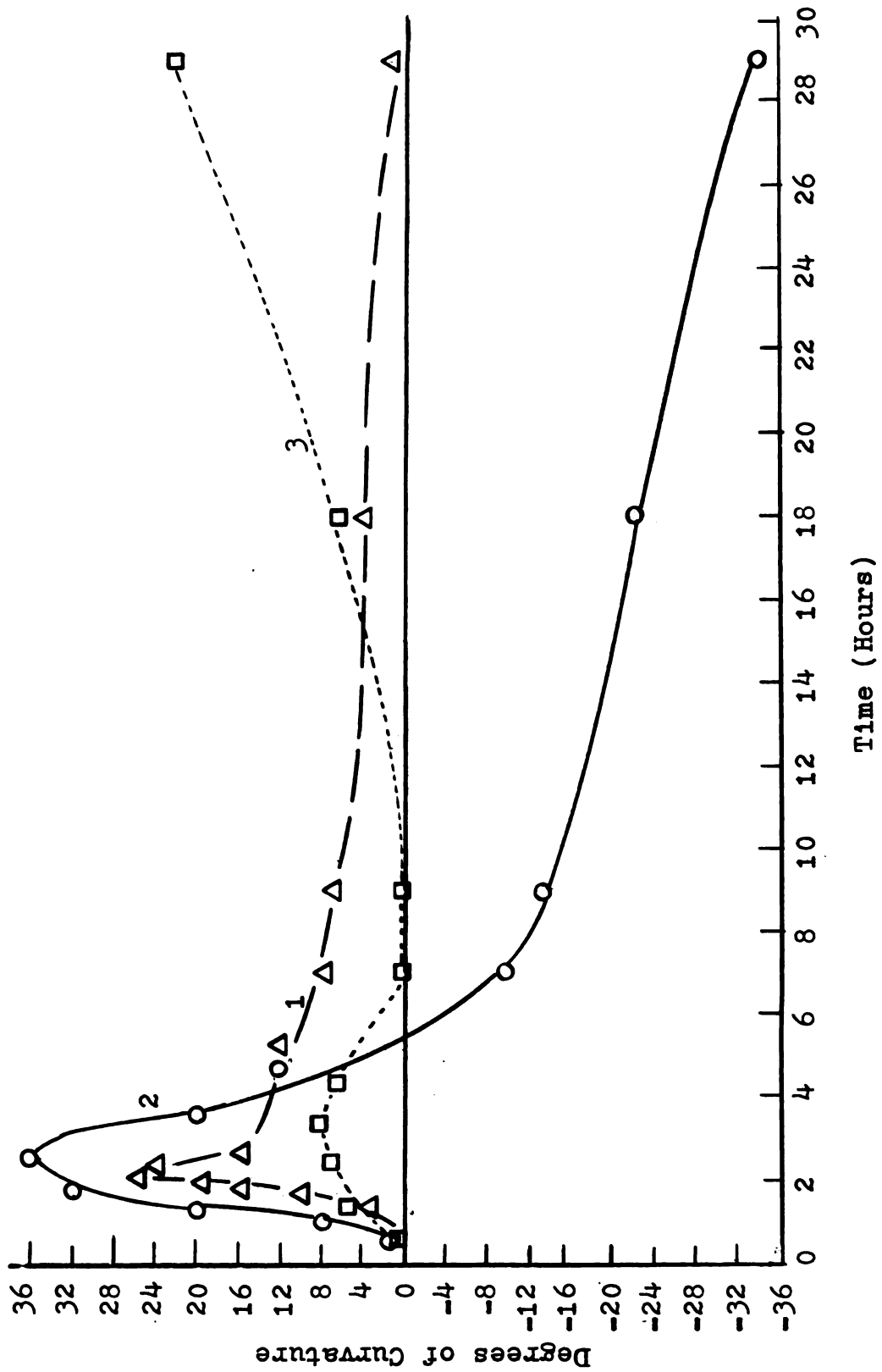
FIGURE 14

Cucumber Curvature Assay

Kinetics of curvature for cucumber seedlings treated with IAA
and 1-iso-propyl IAA: (1) IAA, (2) Left cotyledon iso-Pr IAA,
and right cotyledon IAA, (3) Right cotyledon treated with a
mixture of IAA and iso-Pr IAA.

Time (Hours)

Degrees of Curvature

the expected time course of curvature. In Figure 14, the effect of such treatment is depicted. After the initial effect of indole-3-acetic acid had subsided, a reversal in the direction of bending was seen which persisted for several days.

The following experiments were conducted to determine whether the activity of this alkyl derivative could be attributed to in vivo degradation to indole-3-acetic acid. A 10^{-3} M lanolin solution of indole-3-acetic acid and a 10^{-3} M lanolin solution of 1-iso-propyl indole-3-acetic acid were mixed together to give a concentration of 5 x 10^{-4} M for each compound. When this mixture was applied to one cotyledon, the results shown in curve 3 of Figure 14 were obtained. Apparently the alkylated acid interferes with the normal action of the parent acid. Schlender (83) has shown that indole-3-acetic acid gives nearly the same final response when cucumber cotyledons were treated with concentrations of free acid in the range 10^{-2} to 10^{-4} M. The only difference was that lower concentrations caused a later response. A corresponding slight displacement in time can be seen in curve 3.

Further evidence that the alkylated material was not enzymatically degraded to indole-3-acetic acid was obtained from the observation that a 10^{-5} M solution of 1-iso-propyl-indole-3-acetic acid revealed no spectral change after 24 hours incubation with 3 one cm. cucumber stem sections. If the alkyl chain were removed, a hypsochromic shift would be

expected. Also, if the ring were degraded in any way, one would expect a definite spectral change.

The possibility that branching slows metabolism of this compound and causes the persistent effect was considered. Very similar structures are present in the isomeric butyl analogs. These related indole derivatives were unable to induce any curvature. Such results apparently negate the importance of delayed metabolism unless the slightly smaller iso-propyl group can fit a site which is too small for a butyl group. The definite inhibition exhibited by the tert-butyl derivative in the Avena straight growth assay is good evidence that the molecule remains intact but elicits no auxin response.

An obvious parallel was noted between the Avena straight growth activity of 1-alkylindole-3-acetic acids and Taft's E_s values (100). The E_s values are a measure of the steric effect of various alkyl substituents in ester hydrolysis relative to the methyl group. Even though the values depend upon a transition state (101) they are, however, a reasonable approximation of the relative van der Waals radii. Thus, they might conceivably be correlated with physiological phenomena in which the size of a group is of importance. Schlender (83) noted a similar parallel between activity and the E_s value of an α-substituent on the side chain of indole-3-acetic acid.

As a measure of overall activity, the following fraction was computed for each concentration from 10^{-4} to

10^{-7} M by dividing percent of the control growth of the test compound by the percent of the control growth of indole-3-acetic acid. Those values obtained at 10^{-8} M were too near that of the controls to make them meaningful and were omitted. The average of the four values for each compound multiplied by 100 gave a measure of the activity of the test compound relative to indole-3-acetic acid.

Figure 19 depicts the relative activity of the 1-alkyl-indole-3-acetic acids in the Avena straight growth assay plotted against the corresponding E_s values. If a straight line is drawn from the position of the methyl to that of the tert-butyl group, most of the other compounds correlate with the line in a qualitative sense. At higher concentrations, several of the compounds bearing larger substituents are seen to be inhibitory. The effect of the tert-butyl group is particularly noteworthy (Figure 16).

Another interesting feature of the Avena straight growth curves can be seen in Figure 15. A depression of activity at 10^{-6} M was noted with the lower members of the alkyl indole derivatives. The latter effect may not be significant because the growth response of all of these compounds at lower concentrations does not deviate far from that of the controls. The growth response of the other alkyl derivatives are shown in Figures 16 and 17.

The hydrophilic methyl 1-β-D-glucopyranosyl-indole-3-acetate had essentially no activity in any of the assays. Figure 17 shows that neither enhancement nor antagonism of

FIGURE 15

<u>Avena</u> Straight Growth Assay

Growth curves for <u>Avena</u> coleoptile sections, effect of 1-alky-
lation: (1) IAA, (2) Me IAA, (3) Et IAA, (4) <u>n</u>-Pr-IAA, (5)
<u>iso</u>-Pr-IAA.
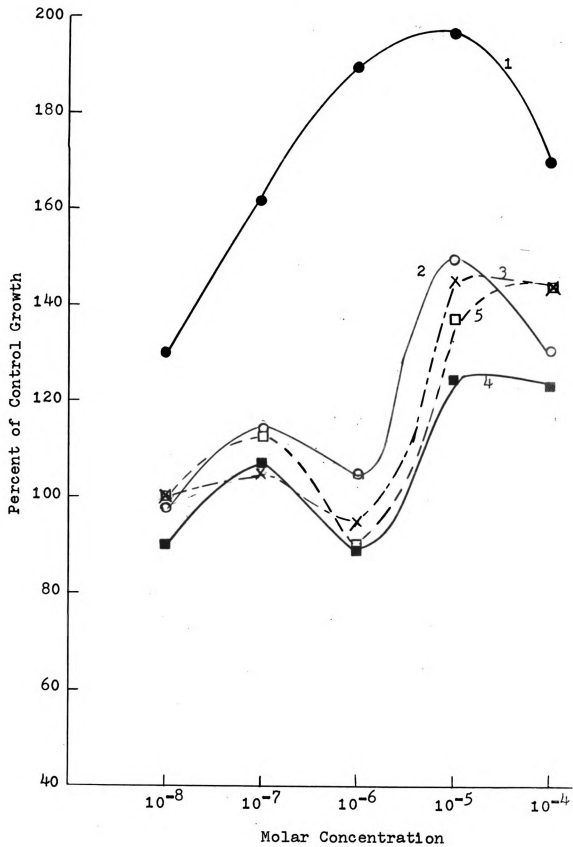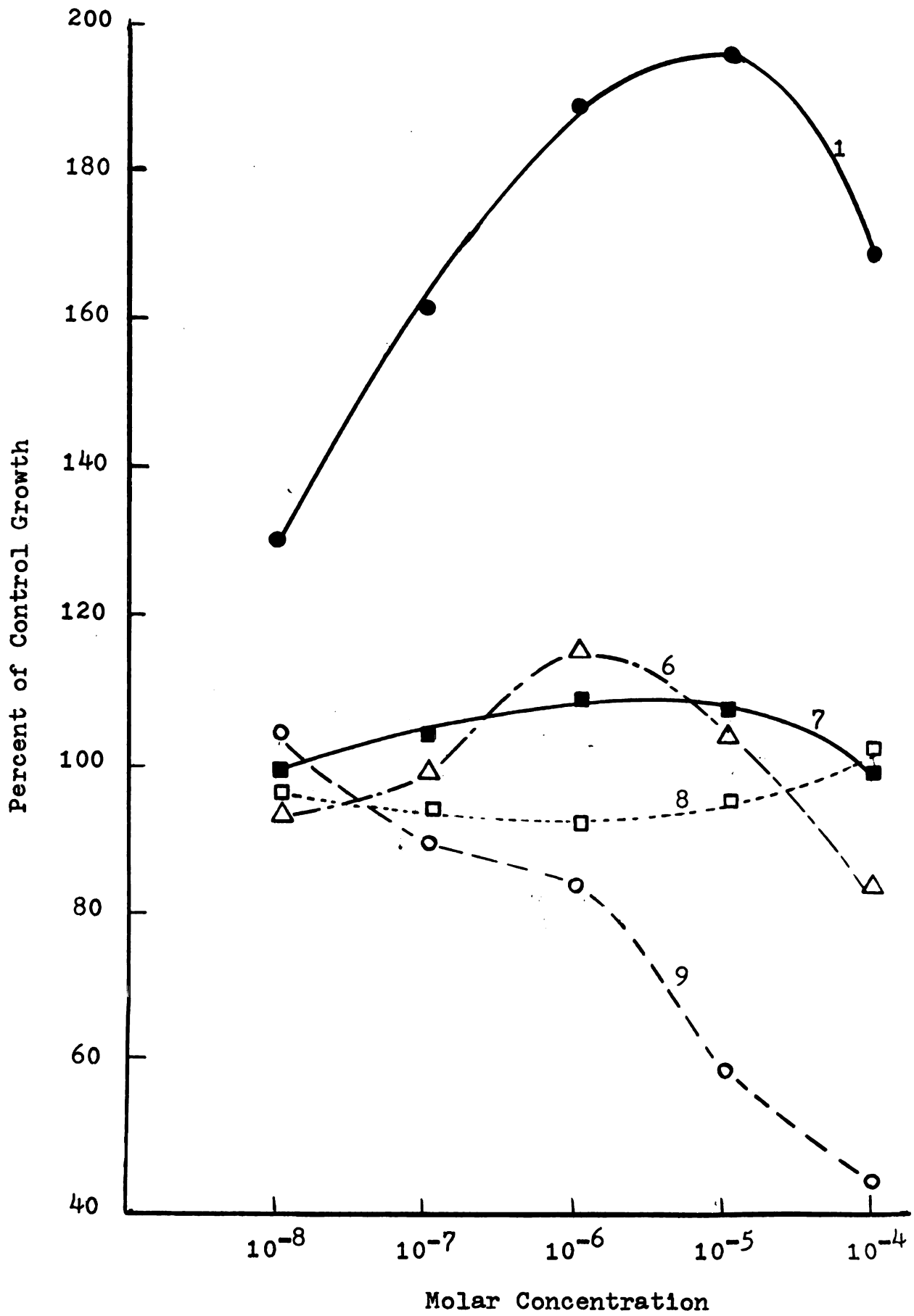
FIGURE 16

<u>Avena</u> Straight Growth Assay

Growth curves of <u>Avena</u> coleoptile sections, effect of 1-alkylation: (1) IAA, (6) n-Bu-IAA, (7) <u>iso</u>-Bu-IAA, (8) <u>sec</u>-Bu-IAA, (9) <u>tert</u>-Bu-IAA.
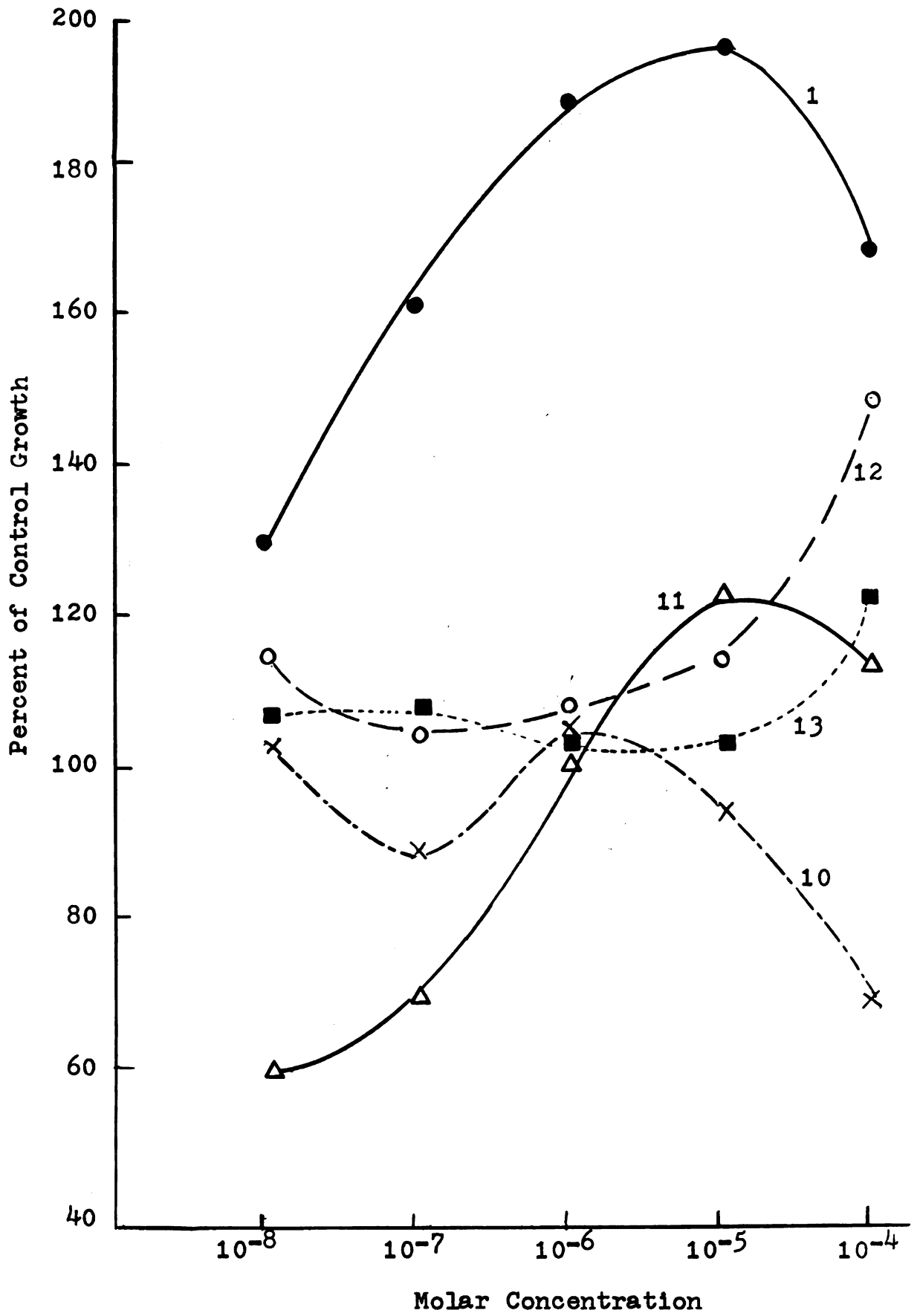
FIGURE 17

Avena Straight Growth Assay

Growth curves for Avena coleoptile sections, effect of 1-alky-lation: (1) IAA, (10) n-Pentyl-IAA, (11) n-Decyl-IAA, (12) n-Octadecyl IAA, (13) Glucosyl MIA.

growth was observed in the Avena straight growth.assay. The latter test is particularly well adapted to show antagonism due to considerable control growth. Equally efficient for demonstrating inhibition is the buckwheat root inhibition test. However, with methyl 1-β-D-glucopyranosylindole-3-acetate both assays showed no concentration dependence and the latter gave almost the same root growth as the control (Figure 17). Figures 10.and 11 show the low level of activity ellicited by this entity in the two other assays.

Veldstra (117) noted that hydrophilic compounds always have a low level of activity. The introduction of a carboxy or hydroxy group on the ring of indole-3-acetic acid greatly lowers the activity of the product. Apparently, the same effect operates with this compound. Hydrolysis surely must not occur or a much higher level of activity would be expected from the product of this hydrolysis, methyl indole-3-acetate.

Another explanation for the lack of both growth enhancement and retardation may be that this molecule cannot penetrate the cell membranes; or if it did so penetrate, the hydrophilic moiety may be so strenuously rejected from the indole-3-acetic acid site that it cannot become attached strongly enough to inhibit the endogenous auxin.

Effect of para-substitution on the benzyl moiety of ethyl 1-benzylindole-3-acetate

The various substituents attached to the benzyl ring of ethyl 1-benzylindole-3-acetate are characterized by a

range of electron withdrawing and donating ability and consequently by a range of Hammett σ_p values. These groups were chosen for investigation in an attempt to correlate this characteristic with biological activity. Hammett's σ_p values (123) are based on the ionization of substituted benzoic acids and hence are a measure of the ability of the substituents to maintain a certain transition state on a moiety attached to the phenyl ring.

Wiberg (123) demonstrated that a good correlation could be drawn between the ionization constants of benzoic acids and those of the phenylacetic acids. The latter compounds are analogous to the benzyl derivatives since they are separated by one methylene group from the charge center in question. When the pKa values of the two series of acids are plotted against each other, a line with a slope of 0.49 is obtained. This indicates that the perterbation energies in the phenylacetic acid series are approximately one-half those of the benzoic acid series (123). The methylene bridge does attenuate the effect of the _para_ group but not to the extent expected.

Porter and Thimann's (75, 76) hypothesis concerning the correlation of activity in auxins with the degree of positive charge on the indolic nitrogen might be conceivably tested with the present series of compounds. Electron withdrawing substituents would be expected to enhance the positive charge on the nitrogen and donating groups would operate in the opposite direction.

The esters of the 1-substituted indole acids were employed because in this way an entire series of compounds could be compared. p-Nitrobenzylindole-3-acetic acid was not obtained in crystalline form and hence all of the acids could not be compared for activity.

In most of the assays all of the 1-benzylindole derivatives had considerably greater activity than did the 1-alkylindole compounds. Figure 18 indicates the relative response of each of the ethyl 1-benzylindole-3-acetate derivatives as compared to the _Avena_ straight growth tests. A higher level of biological activity was noted in this assay than was observed in the other assays. Ethyl 1-benzyl-indole-3-acetate exhibited the greatest biological activity of all compounds tested with the _Avena_ straight growth assay. The fluoro analog had the lowest degree of activity of all of the 1-benzyl derivatives.

The only correlation derived from Hammett's σp value is that which apparently exists for the halo-derivatives. The bromo-substituent has the largest σp value of the three halogen derivatives tested and fluoro- has the smallest. Both the _Avena_ straight growth activity and the Hammett σp values follow a certain trend: $Br > Cl > F$. A similar correlation was also observed in the buckwheat root inhibition assay (Figure 11).

Secondary effects were obviously involved in the _Avena_ response of the other benzyl derivatives. Neither the activity nor Hammett σp values are very different for the methyl

FIGURE 18

<u>Avena</u> Straight Growth Assay

Growth curves of <u>Avena</u> coleoptile sections, effect of <u>para</u>-
substitution on the benzyl moiety of ethyl 1-benzylindole-
3-acetate: (1) IAA, (14) Benzyl EIA, (15) p-Me EIA, (16) p-
F-Benzyl EIA, (17) p-Cl-Benzyl EIA, (18) p-Br-Benzyl EIA,
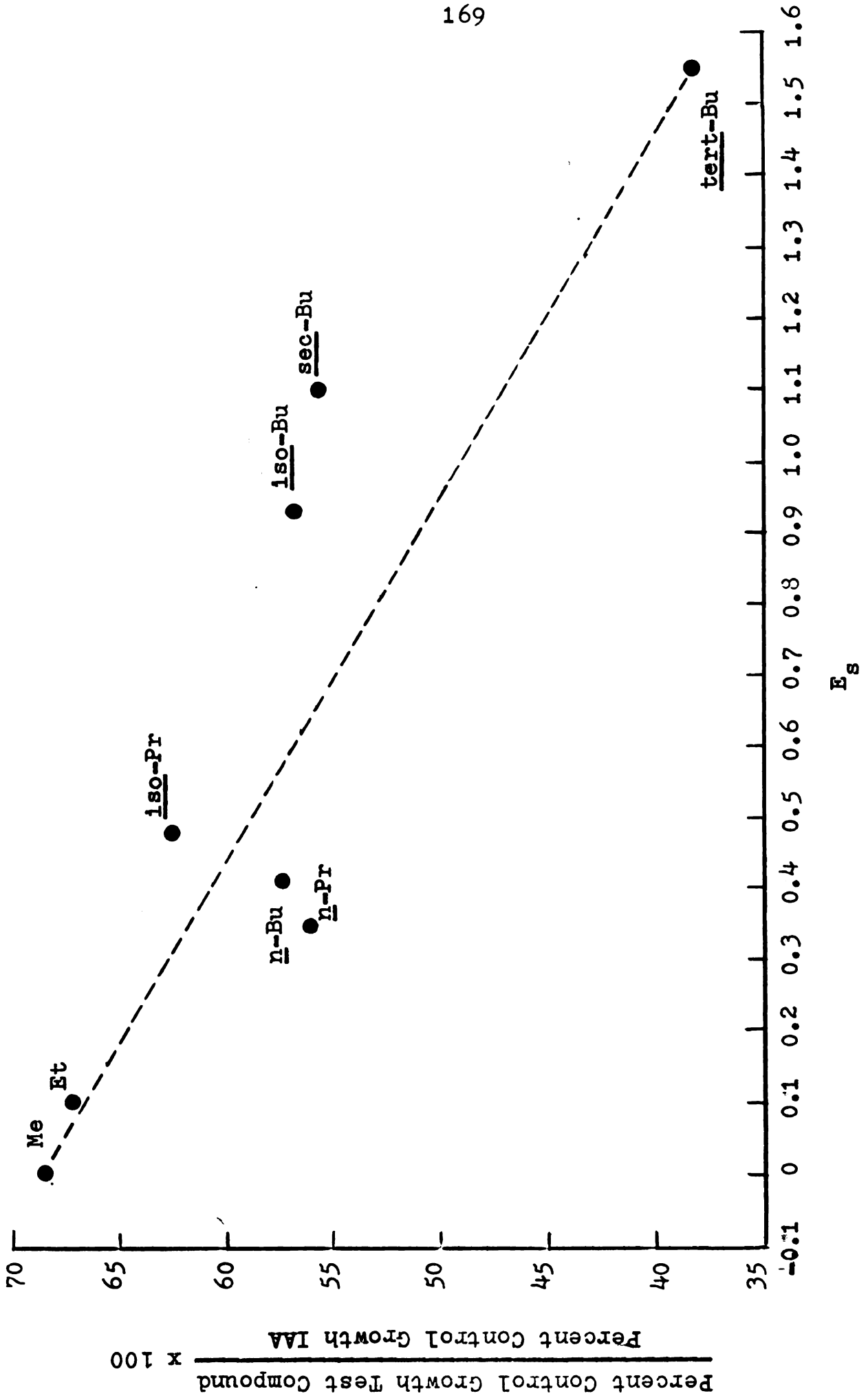(19) p-MeO-Benzyl EIA, (20) p-NO$_2$-Benzyl EIA.
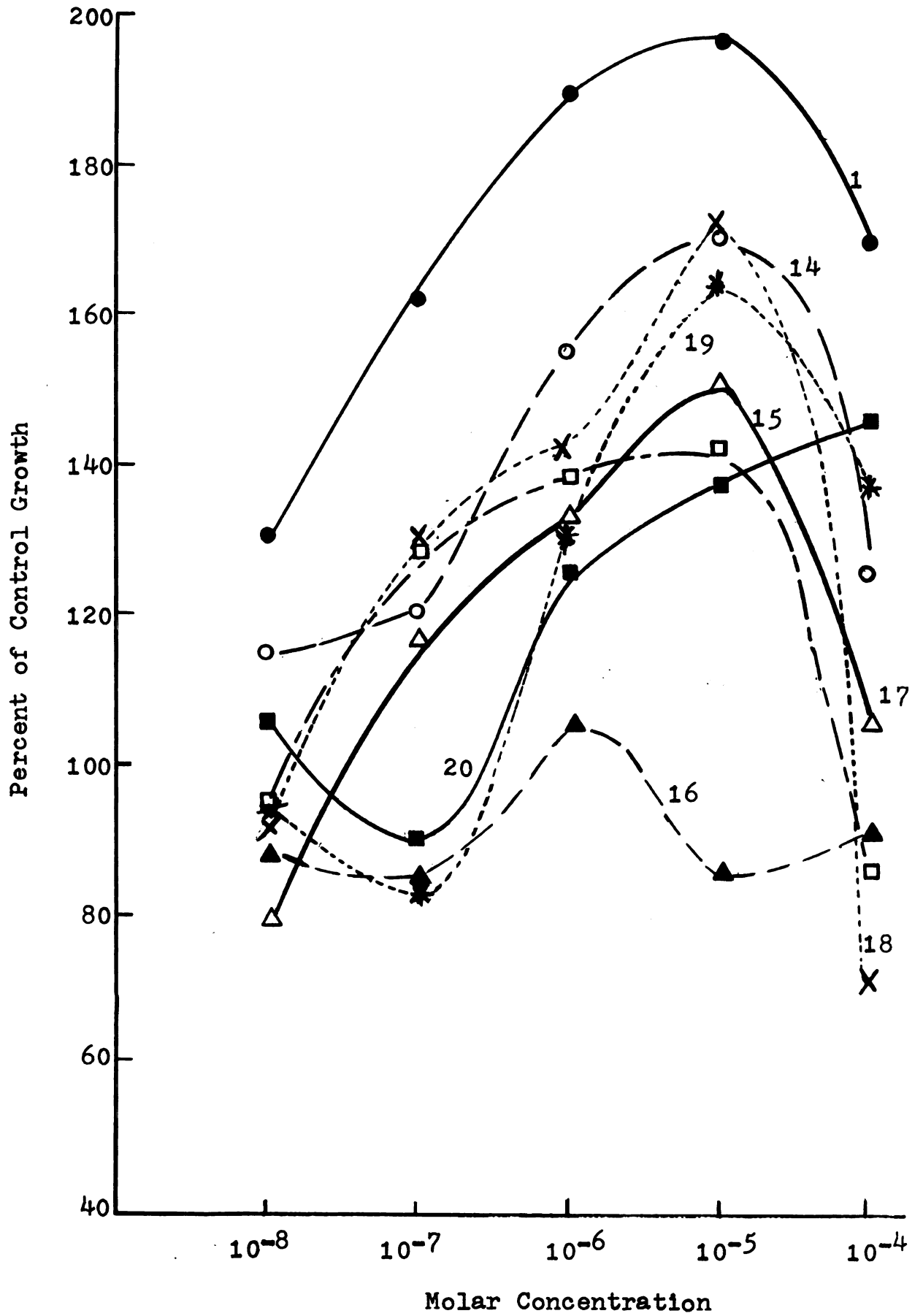
FIGURE 19

Correlation of Taft E_s values with

Avena Straight Growth Activity

Avena straight growth activity of various 1-alkyl indole-3-acetic acids as compared with the Taft E_s values of the substituents.

and methoxy substituents, but the activity of both is disproportionally large if the electronic effect is acting as suggested by Thimann (75). In contrast the nitro substituent, which would be expected to have among the strongest electron withdrawing properties, imparts no higher activity than did the methyl and methoxy substituents.

Hansch and Muir (26) demonstrated that nitro-substituted auxins often have low levels of activity. This may be attributable to polar groups which reduce the lipophilic character of the ring (117). Another possibility is that the anionic character of the nitro group causes the auxin to complete with the attachment site that normally would be occupied by the carboxyl. The latter possibility is in agreement with the finding that nitro groups can replace the carboxyl groups in certain synthetic auxins (117). These results may explain the discovery that the p-nitrobenzyl derivative had no higher activity than did methyl or methoxy compounds. However, the reason for the low activity of the fluoro- compound is not obvious.

The rather high activity observed for ethyl 1-benzyl-indole-3-acetate is noteworthy. para-Substituents do not contribute much to the relative size of the benzyl radical and hence are not expected to interfere significantly with activity on the basis of steric factors alone. None of the withdrawing groups enhanced the activity in this experiment nor did any of the donating groups deduct significantly from that of the unsubstituted benzyl compounds. Therefore, the reasons for the observed activity are not clear.

The finding that ethyl 1-benzylindole-3-acetates did show a level of activity in <u>Avena</u> straight growth approaching that of indole-3-acetic acid lends support to the possibility that the substituted benzoyl analogs assayed by Ritzert <u>et al</u>. (81) are active <u>per se</u>. A given substituted benzoyl moiety has greater electron withdrawing power than does the corresponding benzyl group and would thus be more active according to Thimann's theory. The latter analog of ethyl indole-3-acetate would not be expected to hydrolyze the linkage at the nitrogen atom as easily as would the former and thus, would be more likely to remain intact in the plant. Both the benzyl and benzoyl groups may resist hydrolysis since compounds bearing both groups had reasonably high activity in the <u>Avena</u> test with the acyl compound being more active. In addition, a much more labile compound, methyl-β-D-glucopyranosylindole-3-acetate, had essentially no activity. If the latter compound were hydrolyzed, it would give the high level of activity associated with methyl indole-3-acetate.

The low level of activity noted for all of the benzyl analogs in intact plants may be explained by the inability of these large compounds to penetrate to the site of action.

Another assay on intact plants, the cucumber curvature test, gave a minimal response as is evident in Figure 13. Nevertheless, some tomato ovary growth was noted with this series (Figure 20) indicating that the compounds were transported through an intact membrane to some extent.

FIGURE 20

Tomato Ovary Growth Assay

Effect of various para substituents on the benzyl radical of
ethyl 1-benzylindole-3-acetate on tomato ovary growth ten days
following treatment with 10^{-3} $\underline{M}$ lanolin solutions of test com-
pounds.
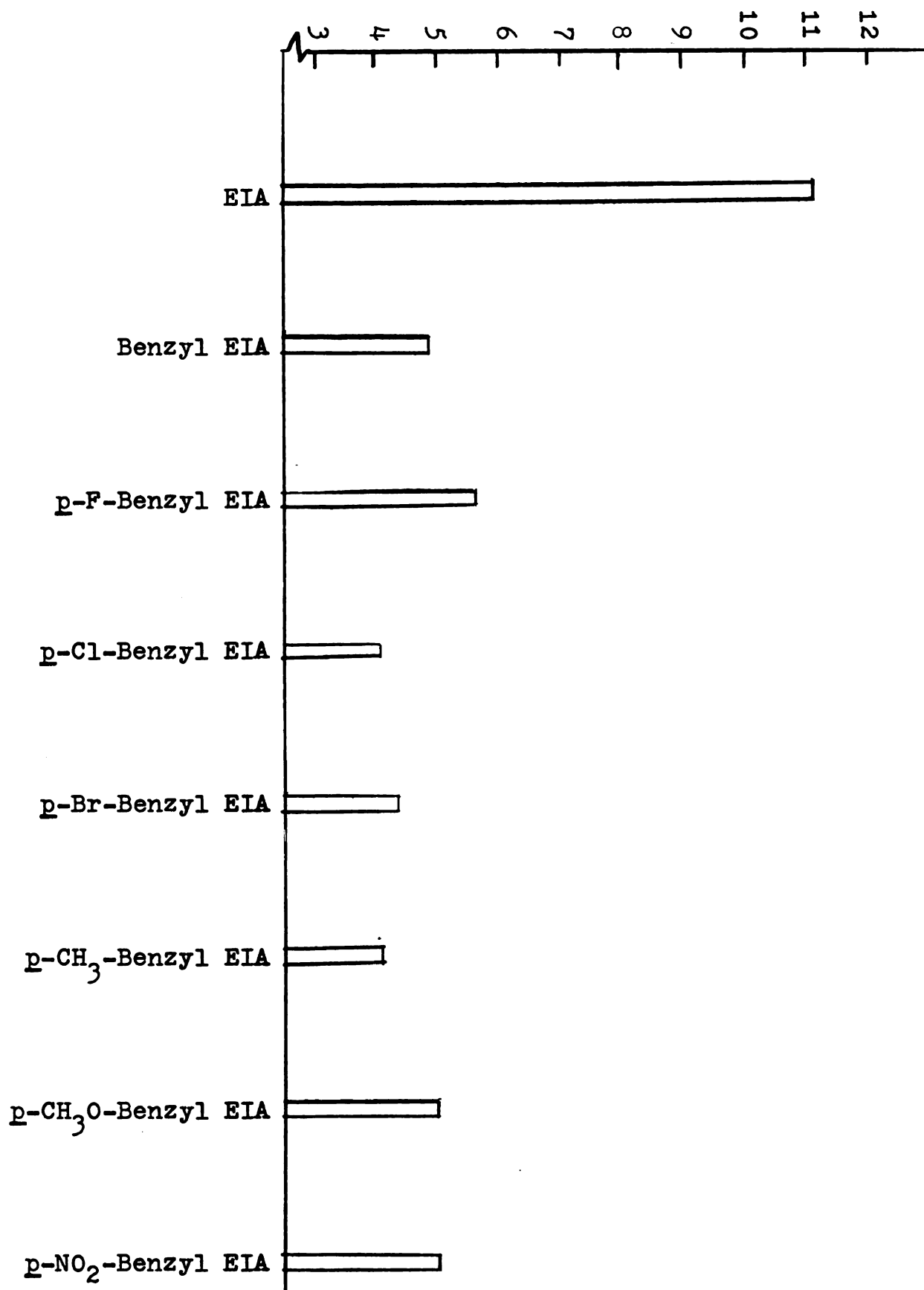
In both the <u>Avena</u> straight growth and bean petiole abscission assays, in which the compounds need not pass through a membrane, a higher level of activity was observed. Figure 10 records the data of the latter experiment. Secondary factors, other than the electronic considerations, must be involved. In this case the nitro group appears to exert an effect that could be attributed to electron withdrawal and an attendant enhancement of the partially positive charge on the indolic nitrogen (75, 76).

Additional evidence of an essentially negative nature was gathered to support the premise that the substituted compounds are active <u>per se</u>. When a 10^{-5} <u>M</u> solution of 1-benzylindole-3-acetic acid was allowed to stand overnight with a few small slices of green tomato fruit no material which migrated as indole-3-acetic acid on thin-layer chromatography could be seen. This is not definite evidence against hydrolysis because the free acid may be degraded <u>in vivo</u>.

To ascertain whether or not 1-substituted derivatives of indole-3-acetic acid acted as antiauxins or antagonists, a 10^{-5} <u>M</u> solution of indole-3-acetic acid was diluted with an equal volume of either 10^{-4} <u>M</u> or 10^{-5} <u>M</u> solutions of several representative indole derivatives. In one experiment, 2.5 ml of 10^{-5} <u>M</u> indole-3-acetic acid was combined with a like volume of 10^{-4} <u>M</u> 1-methylindole-3-acetic acid and in another experiment with 2.5 ml of a 10^{-5} <u>M</u> solution of the substituted acid. Solutions of 1-<u>iso</u>-propylindole-3-acetic acid and ethyl 1-benzylindole-3-acetate were

prepared in a similar manner. Each solution was assayed in the buckwheat root inhibition test. In all cases the level of activity was very nearly that expected of 5×10^{-6} $\underline{M}$ solution of indole-3-acetic acid, thus indicating that the substituted acid or ester did not function as an auxin antagonist by reversing the inhibition established by the free acid.

SUMMARY

SUMMARY

A series of 1-alkyl indole-3-acetic acids were synthesized and assayed for physiological activity. These compounds were made from indoline by way of a substituted indoline. The substituted indoline was dehydrogenated to the corresponding indole with dichlorodicyanobenzoquinone. Attachment of the side-chain ester group to indole was effected with ethyl diazoacetate. Finally the free acids were prepared by saponification of the esters. A list of the indole acids so prepared follows:

1-methylindole-3-acetic acid
1-ethylindole-3-acetic acid
1-n-propylindole-3-acetic acid
1-iso-propylindole-3-acetic acid
1-n-butylindole-3-acetic acid
1-iso-butylindole-3-acetic acid
1-sec-butylindole-3-acetic acid
1-tert-butylindole-3-acetic acid
1-n-pentylindole-3-acetic acid
1-n-decylindole-3-acetic acid
1-n-octadecylindole-3-acetic acid

Methyl 1-β-D-glucopyranosylindole-3-acetate was prepared by an analogous synthetic route and then assayed for biological activity.

Several ethyl 1-p-substituted indole-3-acetates were synthesized from ethyl indole-3-acetate. The latter compounds were assayed by the same methods as were the alkyl analogs. The members of this series are:

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> ethyl 1-benzylindole-3-acetate
> ethyl 1-p-fluorobenzylindole-3-acetate
> ethyl 1-p-chlorobenzylindole-3-acetate
> ethyl 1-p-bromobenzylindole-3-acetate
> ethyl 1-p-methylbenzylindole-3-acetate
> ethyl 1-p-methoxybenzylindole-3-acetate
> ethyl 1-p-nitrobenzylindole-3-acetate

The corresponding acids were prepared from all of the above esters except the nitro-compound.

Ultraviolet and infrared spectroscopy was used to supplement elemental analysis in the characterization of the compounds. Refractive indexes, boiling points, and melting points are reported for all compounds including the synthetic intermediates.

Biological assays were employed to evaluate the activity of the new compounds. Avena straight growth test was the most sensitive measure of activity investigated. Low activity of each compound was noted in the buckwheat root inhibition and tomato fruit-setting assays. The cucumber curvature assay showed essentially no response to any of the compounds tested with the exception of 1-iso-propylindole-3-acetic acid. The latter compound caused delayed bending which was much more persistent than that caused by indole-3-acetic acid.

The bean petiole abscission and the Avena straight growth assays do not require compounds to penetrate intact membranes. Benzyl derivatives were the most active in these tests, indicating that the larger molecules do possess a certain level of activity when they are allowed to reach the site of action.

A correlation was noted between Taft's E_s values and the activity of the lower alkyl compounds. Thus, 1-methyl-indole-3-acetic acid has some activity in the Avena straight growth test and 1-tert-butylindole-3-acetic acid showed definite inhibition. Each of the alkyl compounds showed only a very low level of activity in all of the other assays.

A correlation apparently exists between activity in Avena or buckwheat and Hammett's σp values for the three halo-compounds assayed. Secondary effects apparently over-shadowed the electronic contribution of other para substituents associated with the benzyl esters.

Methyl 1-β-D-glucopyranosylindole-3-acetate was almost entirely inactive in all assays. Such a response is to be expected of very hydrophilic entities.

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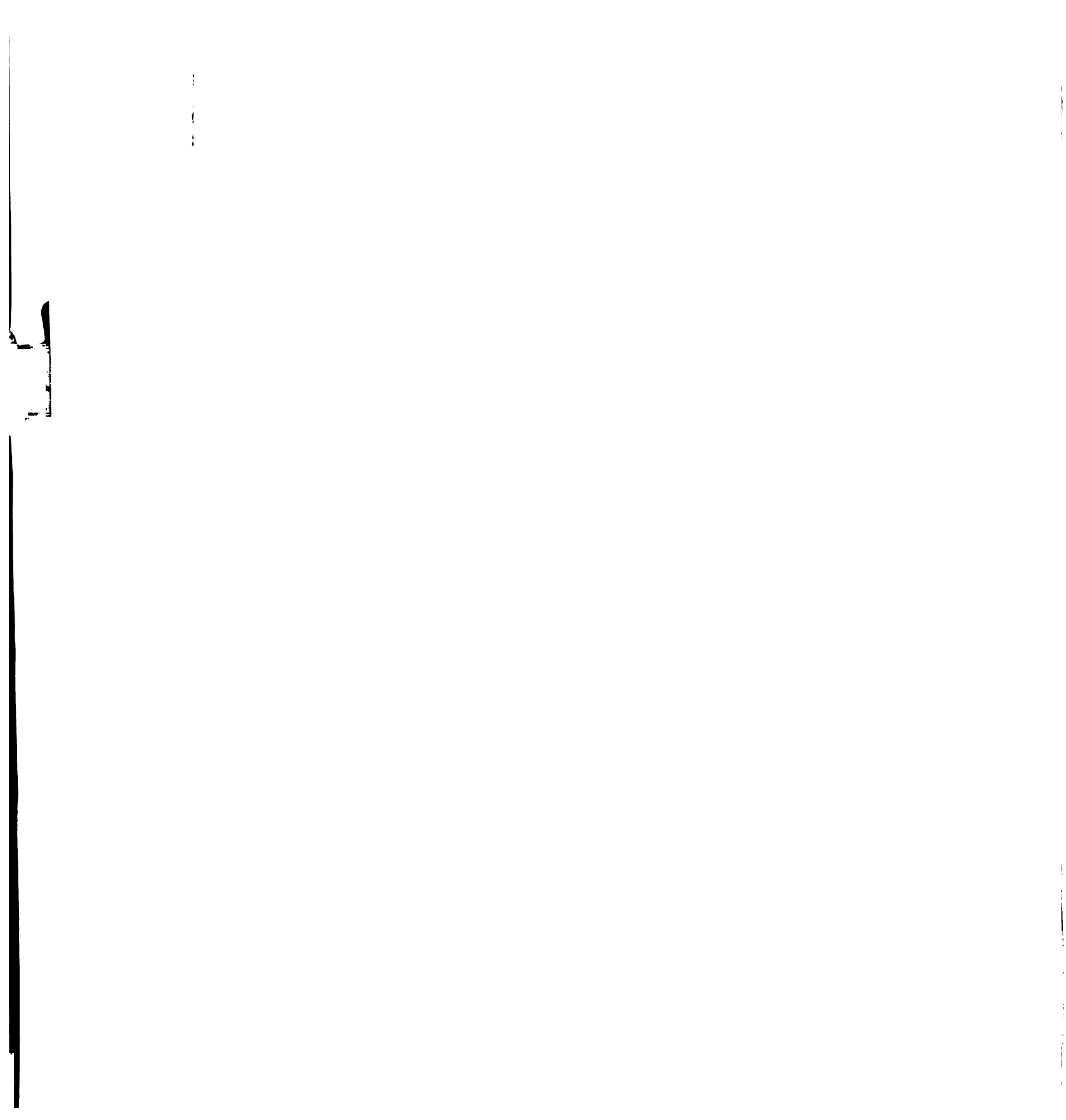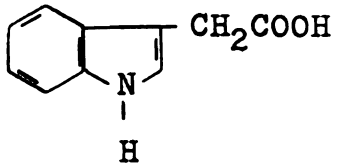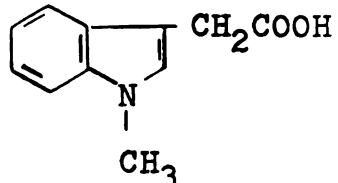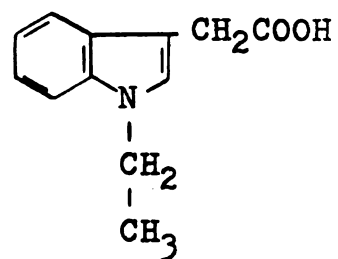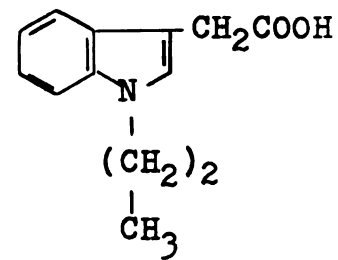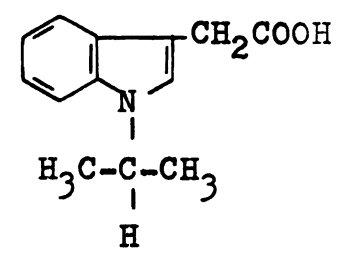
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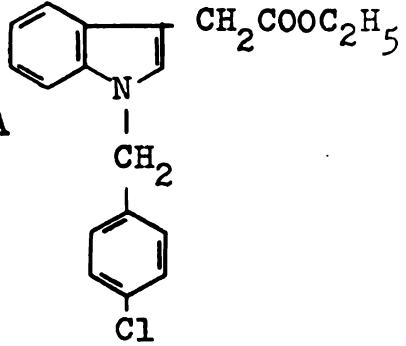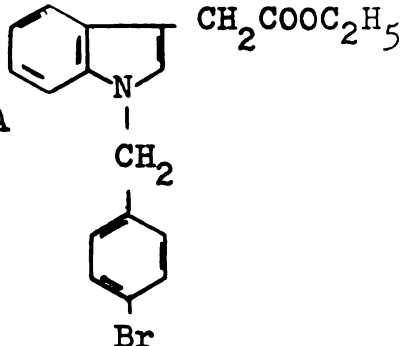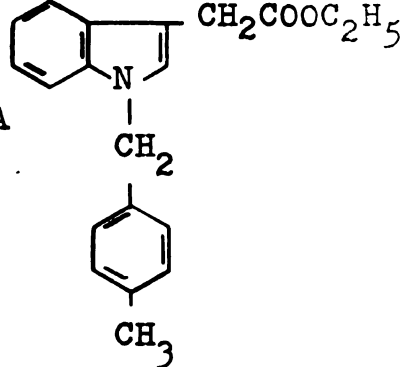
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APPENDIX
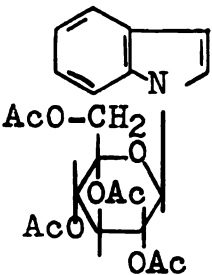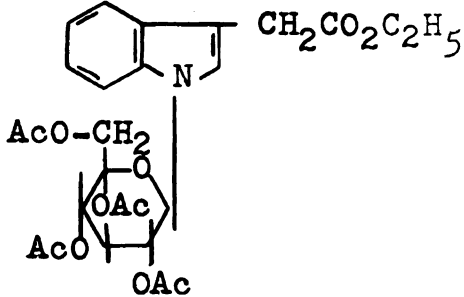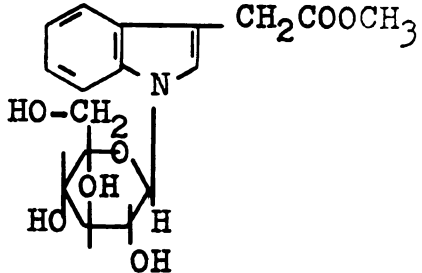
Chemical Name	Abbreviation	Structure
Indole-3-acetic acid	IAA	indole ring with CH_2COOH at position 3, N-H
1-Methylindole-3-acetic acid	Me IAA	indole ring with CH_2COOH at position 3, N-CH_3
1-Ethylindole-3-acetic acid	Et IAA	indole ring with CH_2COOH at position 3, N-CH_2-CH_3
1-normal-Propylindole-3-acetic acid	n-Pr IAA	indole ring with CH_2COOH at position 3, N-$(CH_2)_2$-CH_3
1-iso-Propylindole-3-acetic acid	iso-Pr IAA	indole ring with CH_2COOH at position 3, N-H_3C-C-CH_3 with H

Chemical Name	Abbreviation	Structure

1-normal-Butylindole-3-acetic acid   n-Bu IAA

$$\text{indole-CH}_2\text{COOH}, \quad N\text{-}(CH_2)_3\text{-}CH_3$$

1-iso-Butylindole-3-acetic acid   iso-Bu IAA

$$\text{indole-CH}_2\text{COOH}, \quad N\text{-}CH_2\text{-}CH(CH_3)_2$$

1-secondary-Butylindole-3-acetic acid   sec-Bu IAA

$$\text{indole-CH}_2\text{COOH}, \quad N\text{-}CH(CH_3)\text{-}CH_2\text{-}CH_3$$

1-tertiary-Butylindole-3-acetic acid   tert-Bu IAA

$$\text{indole-CH}_2\text{COOH}, \quad N\text{-}C(CH_3)_3$$

1-normal-Pentylindole-3-acetic acid   n-Pentyl IAA

$$\text{indole-CH}_2\text{COOH}, \quad N\text{-}(CH_2)_4\text{-}CH_3$$

Chemical Name	Abbreviation	Structure
1-normal-Decylindole-3-acetic acid	n-Decyl IAA	Indole with CH_2COOH at 3-position and $(CH_2)_9CH_3$ on N
1-normal-Octadecylindole-3-acetic acid	n-Octadecyl IAA	Indole with CH_2COOH at 3-position and $(CH_2)_{17}CH_3$ on N
Ethyl indole-3-acetate	EIA	Indole with $CH_2COOC_2H_5$ at 3-position and H on N
Ethyl 1-benzylindole-3-acetate	Benzyl EIA	Indole with $CH_2COOC_2H_5$ at 3-position and CH_2-phenyl on N
Ethyl 1-(para-fluorobenzyl)-indole-3-acetate	p-F-Benzyl EIA	Indole with $CH_2COOC_2H_5$ at 3-position and CH_2-(4-F-phenyl) on N

Chemical Name	Abbreviation	Structure
Ethyl 1-(<u>para</u>-chlorobenzyl)- indole-3-acetate	<u>p</u>-Cl-Benzyl EIA	
Ethyl 1- (<u>para</u>-bromobenzyl)- indole-3-acetate	<u>p</u>-Br-Benzyl EIA	
Ethyl 1-(<u>para</u>-methylbenzyl)- indole-3-acetate	<u>p</u>-Me-Benzyl EIA	

Chemical Name	Abbreviation	Structure
Ethyl 1-(para-methoxy-benzyl)-indole-3-acetate	p-MeO-Benzyl EIA	
Ethyl 1-(para-nitro-benzyl)-indole-3-acetate	p-NO$_2$-Benzyl EIA	
1-(2',3',4',6'-Tetra-O-acetyl-β-D-gluco-pyranosyl) indoline	Indoline TAG	

Chemical Name	Abbreviation	Structure

1-(2',3',4',6'-Tetra-
O-acetyl-β-D-gluco-
pyranosyl) indole

Indole TAG

Ethyl 1-(2',3',4',6'-
Tetra-O-acetyl-β-D-
glucopyranosyl)
indole-3-acetate

EIATAG

Methyl 1-β-D-glucopyran-
osylindole-3-acetate

Glucosyl MIA