



New plant growth-regulators
by Jack R Gaines

A THESIS Submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree
of Doctor of Philosophy in Chemistry
Montana State University
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Abstract:

A comprehensive review and discussion of known synthetic plant growth-regulators is given. Two main series of compounds, the substituted phenylhydrazono-acetic and propionic acids, were synthesized and tested for activity as plant growth-regulators. The parent acids, phenylhydra-zonoacetic acid and α -phenylhydrazonopropionic acid, were found to be highly active. All of the substituted derivatives were considerably less active and in most cases were inhibitory.

α -Phenyliminopropionic acid, ethyl β -(p-chlorophenylamino)-acrylate, and ethyl β -(p-methylphenylamino)-acrylate were also prepared and tested. All three of these compounds, with the possible exception of the p-chloro-compound, were inactive. 2-Nitro-4-methoxyphenoxyacetic acid was prepared and tested in order to test a hypothesis proposed by the author.

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I. ABSTRACT

A comprehensive review and discussion of known synthetic plant growth-regulators is given. Two main series of compounds, the substituted phenylhydrazono-acetic and propionic acids, were synthesized and tested for activity as plant growth-regulators. The parent acids, phenylhydrazonoacetic acid and α -phenylhydrazonopropionic acid, were found to be highly active. All of the substituted derivatives were considerably less active and in most cases were inhibitory.

α -Phenyliminopropionic acid, ethyl β -(p-chlorophenylamino)-acrylate, and ethyl β -(p-methylphenylamino)-acrylate were also prepared and tested. All three of these compounds, with the possible exception of the p-chloro-compound, were inactive. 2-Nitro-4-methoxyphenoxyacetic acid was prepared and tested in order to test a hypothesis proposed by the author.

II. INTRODUCTION

In 1938 Koepfli, Thimann and Went (56) published the first comprehensive report on synthetic plant growth-regulators. From their experimental evidence, they concluded that the minimum structural requirements for cell elongation activity in higher plants are: (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 group) on this side chain at least one carbon atom removed from the ring, and (e) a particular space relationship between the ring and the carboxyl group. Following this structural requirement, Zimmerman and Hitchcock (126) tested a series of phenoxyacetic acids and benzoic acids and found in the case of certain halogen derivatives remarkable growth regulating activity. The most promising of these compounds being 2,4-dichlorophenoxyacetic acid (2,4-D). Up to the present time, this compound and its derivatives have remained the most active of all synthetic growth-regulators.

Since this work of Zimmerman and Hitchcock, a large and diverse group of organic compounds has been reported to regulate plant growth. Many of the determinations, however, do not measure the accelerating effects on growth, but the inhibition of growth or toxicity effects. Such evidence does not suffice to identify the substance as growth regulators which may either increase or inhibit growth depending upon their concentration.

With the exception of a few isolated compounds only the following series of compounds have shown promise as true growth-regulating compounds: the benzoic acids, the phenoxyalkylcarboxylic acids, the thio-phenoxyalkylcarboxylic acids, the naphthoxyalkylcarboxylic acids, the indolealkylcar-

boxylic acids, and the phenylalkylcarboxylic acids. Of these series, only the benzoic and phenoxyacetic acids have received considerable attention; especially from the standpoint of relating chemical structure with physiological activity.

The purpose of this research was, through structural analogy, to propose and synthesize a new series of compounds having growth-regulating properties.

III. HISTORICAL AND THEORETICAL DISCUSSION

Chemical Nature of the Auxins

Bonner and Wildman (18) indicated in 1946 that indoleacetic acid (IAA) is a principle native auxin in higher plants. With the development of chromatography, countercurrent distribution, and spectrophotometric micro-methods, the identification and estimation of plant growth substances have been extended (97,123,98,35,66,99).

In at least four instances, the growth substance activity of a plant concentrate has been found to reside in part in materials not identical to IAA. In all instances, however, the material in question has turned out to be either a derivative of IAA or closely related to it. The first of these was the case of the neutral growth substance described by Larsen (61). This was identified as indole acetaldehyde and is active only after conversion in the plant to IAA (62). A second case is that of the fruit setting factor contained in immature corn kernels (85). This substance, which is reportedly 100 times more active than IAA, was shown to be the ethyl ester of IAA (85). Thirdly, in the countercurrent distribution of the acidic fraction of cabbage leaves, Holley et.al. (50) found, in addition to IAA, two separable although minor components, both active in the Avena curvature test. Although the chemical nature of these substances has not been established, both give the Salkowski reaction and are possibly related to IAA in some manner. Finally, Jones et.al. (54) isolated indoleacetonitrile from plant tissue and it was found to be more effective as an auxin for Avena coleoptile tissue than IAA (12). Stowe and Thimann (93) have reported, however, that IAA can be detected by paper chromatography in solutions

of indoleacetonitrile on which *Avena* coleoptile sections grow for 24 hours as well as in extracts of the coleoptile tissue, thus indicating that this tissue converts the nitrile to the acid. Thimann (103) reports that the nitrile is inactive in the pea test, but the ether extract of coleoptile sections grown for 48 hours on a solution of the nitrile causes a response in the pea test which would indicate a conversion of more than 50% by the coleoptile tissue. Thus there is still no strong evidence to indicate the presence of any native growth substance other than IAA.

Methods of Testing

The principle difficulty in appraising all the literature pertaining to chemical structure and plant growth activity lies in the correlation of data derived from many different physiological responses. Of all the responses used, such as induction of parthenocarpy (67,130), organ modification (97,129,131), control of abscission (118), initiation of roots (46, 127), cell elongation (16,56,13,17,72), inhibition of root growth (2,14,20, 36,45,84,95) and seed germination (28), the stimulation of cell elongation has been used most extensively.

Due to the variety of tests applied to various compounds, many false conclusions have been drawn by mixing results obtained with different test methods. This discussion will contain primarily the results of work obtained by using a cell elongation method since only by the use of homogeneous material can conclusions be safely drawn.

The *Avena* straight growth (15,13,71,72,24) and the pea test (87,100, 80,18) are the two preferred methods since secondary factors (penetrability, polar transport, inactivation of compounds) are excluded to a large extent.

However, the complex nature of the pea test as well as other features (24) make it less satisfactory than the Avena straight growth method.

Norman and Weintraub (76) have clearly outlined the limitations of existing data as determined by the variations in test objects, different experimental conditions and techniques, etc., thus many of the available data possess only a semi-quantitative character.

In expressing the results of a certain test method in absolute terms, it must be remembered that the response to stimulation, which becomes an inhibition at higher concentrations, is described by an activity curve with a maximum. It is obvious then, that two compounds may have their maximum response at different concentrations. However, as Veldstra (113) has indicated, relative activities are often more important than absolute ones in studying structure-activity relationships and therefore it is essential that the activities of compounds under comparison be determined simultaneously under identical conditions.

Structure and Activity

The correlation between chemical structure and physiological or pharmacological activity has always been fascinating because it may have a double purpose: a basic pattern for synthetic work aiming at new compounds of comparable or enhanced activity and a possible insight as to the mode of action. However, due to the complexity of the structure of the active compounds and the test methods used in assessing activity, little or no correlation can be made. Plant growth substances, however, appear very attractive for this kind of analysis because both the structures and test methods are relatively simple.

In 1938 Koepfli, Thimann and Went (58) published the first comprehensive report on synthetic plant growth-regulators. They concluded from their work that the minimum structural requirements for growth activity in higher plants are: (a) a ring system as a nucleus, (b) a double bond in this ring, (c) a side chain, (d) a carboxyl group (or a structure readily converted to a carboxyl group) on this side chain at least one carbon atom removed from the ring, and (e) a particular space relationship between the ring and carboxyl group.

In subsequent years a large number of compounds have been screened for activity and from the results obtained, restatements of the structural requirements in different forms have appeared. Several reviews of these developments have been published (113,74,101,81,102). For the purpose of discussion, certain of these requirements will be repeated here.

As more compounds became known, the requirements as stated by Koepfli et.al. fell short of accounting for all obtained results. Veldstra (106, 107) restated the requirements for a plant growth substance as follows: (a) the substance should have a basal ring system with high surface activity, and (b) it should have a carboxyl group in a very definite spatial position with respect to this ring system.

Later on Veldstra and Booi (108) formulated the requirements in more detail as: (a) a basal ring system (nonpolar part) with high interface activity and (b) a carboxyl group (polar part), in general a group of acidic character, in such a spatial position with respect to the ring system, that on absorption of the active molecule to a boundary, this functional group will be situated as peripherally as possible. Their theory is essentially

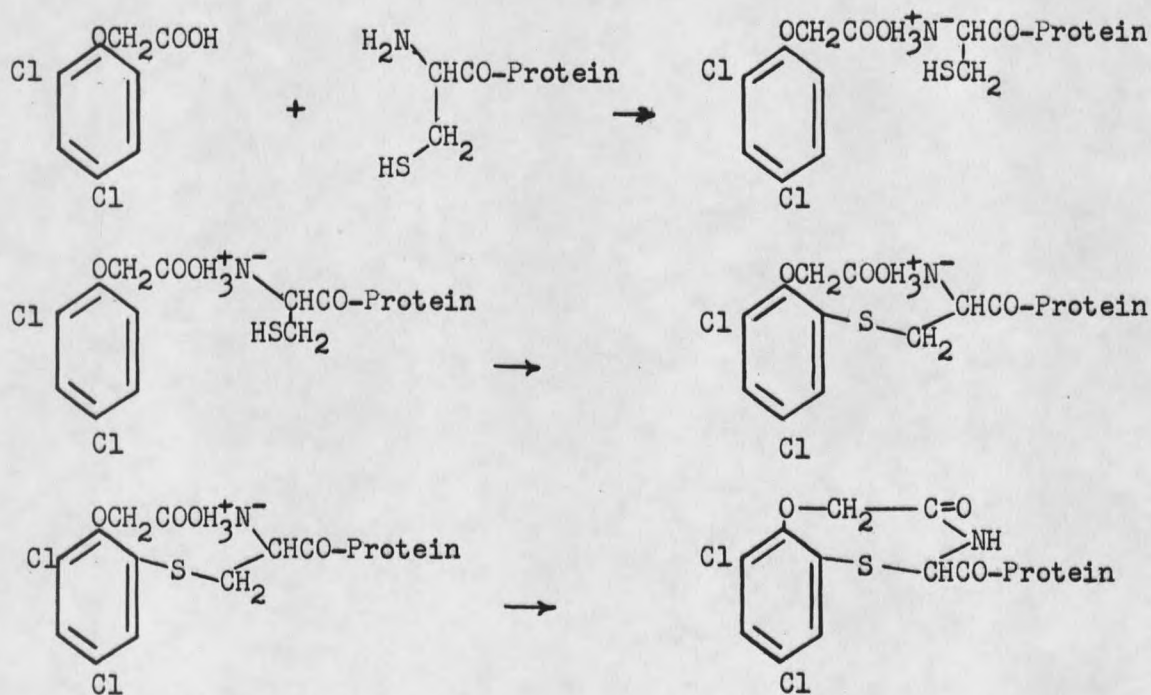
one of altered permeability of cell membranes caused by the adsorption of growth-controlling compounds to this phase boundary. The theory was based mainly on a polarographic investigation in which the effects of the compounds on the oxygen maximum were used as a measure of the adsorbability of the compounds and therefore of their physiological activity. Paleg and Muir (81) point out that this theory falls far short of accounting of the activity or non-activity of many compounds, and conclude that chemical reactivity is more important than adsorbability.

Veldstra (113) further proposed that for positive activity, a non-coplanarity exist between the carboxyl group and the ring system. The conclusion resulted from the spectacular difference in physiological activity between the cis and trans-cinnamic acids (106) (the cis form being active). Only the trans form tends to occur in a flat form (113). The same results have been found in the case of cis-trans tetralideneacetic acid and naphthalene-1-acrylic acid (106).

In the opinion of the author, the most promising interpretation as to the mode of action of growth-regulators and the structural requirements necessary for activity has been proposed by Hansch and Muir (72,40,39,74). They first proposed the "ortho effect" (39), stating that compounds must have a free or potentially free ortho position to be active in cell elongation. In 1950, Muir and Hansch (72) listed the minimum structural requirements for growth activity as: (a) an unsaturated ring (preferably aromatic), (b) a carboxyl group or a group capable of conversion to a carboxyl group (this group may be attached through a chain of one or more atoms or directly to the ring), (c) at least one of the positions on the

ring ortho to the point of attachment of the carboxyl group or the side chain carrying the carboxyl group must be capable of reaction with an electron-rich plant substrate (the ortho position must hold the proper electron density and the group or atom at that position must be capable of displacement under the conditions of reaction), (d) according to Veldstra and Booij (108) the relative lyophilic character of the molecule compared to the hydrophilic nature of certain attached groups such as the carboxyl group may effect the activity of a compound as a growth-regulator, and (e) characteristics such as the relation of the side chain carboxyl group to an ortho position may be of importance in determining the activity of a compound, but little is known about them.

In 1951 Hansch, Muir and Metznerberg (40) postulated the two-point substrate reaction:



This mode of action is supported by considerable evidence which will be given in detail in the discussion of the phenoxyacetic acids.

Since several requirements formulated for activity pertain to a special group of growth substances, they will be discussed with the respective types of compounds.

The Ring System

The presence of a ring system appears to be an unquestioned requirement for activity. Up to the present time, no active compound has been found lacking a ring nucleus. Also, up to now no active compound has been found which lacks at least one double bond within the ring; when only one is present (as in cyclohexene) a position of the side chain next to the double bond seems to be necessary for activity (121).

Veldstra (113) considers the area of the ring system as being important and lists the naphthalene or indole nucleus as being most favorable in size. He suggests that this size is equalled to some extent by certain substituted benzene nuclei, e.g. 2,4-dichlorophenol. The inactivity of such compounds as 2,6-dichlorophenoxyacetic acid (96,129) and 2-fluoro-4-methylphenoxyacetic acid (119) as compared to the very high activities of 2,4-dichlorophenoxyacetic acid and 2-methyl-4-fluorophenoxyacetic acid (119) would seem to indicate that the area of the ring system could at most be of minor importance in the structure of active compounds.

The Polar Group in the Side Chain

The presence of a carboxyl group in the side chain appears to be essential for activity. Replacement of the carboxyl group by SO_3H generally results in inactivity or at the most only slight activity. 2-(1-Naphthalene)

-ethanesulphonic acid (106), and 1-naphthalene-sulfuric acid (113) have been shown to be inactive. Indole-3-methanesulphonic acid (113) and indolyl-sulphuric acid (106) show slight activity; the activity of the former has been questioned however (122). Sodium 2-(2,4-dichlorophenoxy)-ethyl sulfate (114,47,57) is the only non-carboxylic compound which has an activity comparable to that of carboxylic compounds.

The Side Chain

That the carboxyl group must be separated from the ring system by at least one carbon atom (Koepli et.al.), can no longer be held as a structural requirement. The abandonment of this requirement resulted when the high activity of 2,3,6-trichlorobenzoic acid (13) became known. Since this initial discovery, many substituted benzoic and naphthoic acids have been shown to be active.

Synerholm and Zimmerman (96) observed an oscillation in the activity of ω -(2,4-dichlorophenoxy)-alkylcarboxylic acids and ω -(β -naphthoxy)-alkylcarboxylic acids; the acetic and butyric acids were active, the propionic acid inactive. These authors suggested that the higher homologues would not be active per se but, being subject to a β -oxidation, would produce the active 2,4-dichlorophenoxyacetic acid only when possessing an even number of carbon atoms in the side chain. This reasoning has been supported by Fawcett et.al. (29), who found evidence of β -oxidation in the series of ω -phenoxyalkylcarboxylic acids in flax plants.

Substitution of a methyl group in the α -position of a phenoxyacetic acid has little effect, while a phenyl group or di-methyl substitution greatly decreases activity. Wain et.al. (89,88) consider the alpha hydrogen

atom as being essential to activity. Due to the activity of the benzoic and naphthoic acids, this author prefers to consider the α -substitution effect as a steric factor.

Phenylacetic Acid and Related Compounds

Phenylacetic acid has a relatively high activity as compared to the nearly inactive phenoxyacetic acid. Di- α substitution in the side chain completely inactivates the compound (1). This definitely seems to be a case of steric effects as seen from the following evidence: dl α -n-propylphenylacetic acid and dl α -allylphenylacetic acid are more active than the parent phenylacetic acid, while the branched chain compound, dl α -isopropylphenylacetic acid, is almost inactive (109).

Thimann (102) concluded that the introduction of a nitro group into the ring of phenylacetic acid abolishes activity since he found both the 4-nitro- and 2,4-dinitro- derivatives to be inactive. He proposed that this substituent, being highly deactivating to the benzene ring, lowers the binding capacity of the molecule. Veldstra (113) has, however, found the 2-nitro- and 3-nitro-derivatives to have an activity equal to or greater than the parent compound, and stated that Thimann's generalization was not justified. The -T effect of the nitro group offsets the +T effect of the carboxymethoxy group in the order of para>ortho>meta; this is in reference to the proposed bonding at an ortho position to the carboxylic side chain. This order is the same as that observed for the activities of the three isomers.

This relationship of position to activity is similar for the benzoic and phenoxyacetic acids and will be discussed later in more detail.

As stated previously, only cis-cinnamic acid (106) possesses activity. Nivard (113) investigated several substituted cis-cinnamic acids. It was found that the 2 and 4-chloro compounds had an enhanced activity, the 4-chlorocinnamic acid being the more active. Both 2- and 4-nitrocinnamic acid were inactive.

Cis-1-phenylcyclopropane-2-carboxylic acid was found to be active and like the cinnamic acids, the trans isomer was inactive (111):

Indole-3-alkylcarboxylic Acids

Substitution in the pyrrole nucleus strongly reduces activity in indole-3-acetic acid (48,91,92). This would indicate that a free position 2 is essential for activity. Substitution in the benzene ring of the indole nucleus decreases only slightly and may even enhance activity. The 4-,5-, and 6-chlorindole-3-acetic acids (48) have activities equal to or greater than the parent acid. Substitution in position 7, however, generally causes a decrease in activity, particularly if the indole-3-acetic acid is already substituted elsewhere.

The apparent importance of positions 2 and 7 (adjacent or ortho to the NH group) recalls the importance imposed upon the ortho position in the phenoxyacetic acid series (Hansch and Muir (39)).

Koepfli et.al. (58) studied the effect of substitution in β -indole-3-propionic acid and γ -indole-3-butyric acid. These parent acids were found to have high activities in the pea test. The 2-carboxy derivatives and the 5-,6-, and 7-methoxy derivatives of the propionic acid were all inactive. As mentioned above, this inactivation does not occur with indole-3-acetic acid; 5-,6-, and 7-methoxyindole-3-acetic acids are quite as active as the

parent acid (31).

Phenoxy Compounds

The practical importance of 2,4-dichlorophenoxyacetic acid (2,4-D) has caused extensive investigations on the effects of nuclear substitution in phenoxyacetic acid. Nuclear substitution studies of some of the higher homologues have been conducted, but to a lesser degree. Most of the correlations between physiological activity and chemical structure have resulted from these studies of the phenoxyacetic acid compounds. As will be seen later, the substituted benzoic acids have also received considerable attention.

Bonner (15) suggested that the relative growth promoting activities of the substituted phenoxyacetic acids might be accounted for by the differences in the degree of ionization of the acids. However, Muir et.al. (71) and Hayes and Branch (41) have shown that no such relationship occurs.

Leaper and Bishop (64) in 1951 prepared all of the mono-, di-, and trichlorophenoxyacetic acids and tested their effect on tomato plants and as inhibitors of growth of *Lupinus albus* seedlings. Muir et.al. (71,73,72) and Thimann (102) obtained comparative activities for a number of the compounds. Leaper and Bishop (64) summarized their results in a table (Table I) in which the most active compound (in cell elongation) was rated as 100. Because of the inactivity of the 3,5-, 2,3,5-, and 3,4,5- acids in these tests they proposed that an open position para to an open ortho position is required for activity and the active molecule is involved in the formation of quinoid compounds in plant cells. Wain and Wightman, however, examined these same compounds for activity in the straight growth of *Avena coleoptiles*

TABLE I

Relative Activities of Chlorophenoxyacetic Acids in Tomato Test (64)

Position of chlorine groups in nucleus	Activity in cell elongation (epinasty, etc.)	Activity in rooting intact stems
2	2.0	10.0
3	10.0	12.5
4	10.0	25.0
2,3	1.0	5.0
2,4	66.6	77.5
2,5	100.0	100.0
2,6	0.0	0.0
3,4	20.0	10.0
3,5	0.0	0.0
2,3,4	1.0	0.0
2,3,5	0.0	0.0
2,3,6	0.0	0.0
2,4,5	40.0	250.0
2,4,6	0.0	0.0
3,4,5	0.0	0.0

and in the pea test (117). They found in both tests that the 2,3-, 2,3,4-, 2,3,5- and 3,4,5-acids were active. It is seen that these compounds do not comply with the hypothesis of Leaper and Bishop and yet are active.

Osborne and Wain (77,78,79) studied a series of aryloxyacetic acids in different tests (including straight growth and the pea test) and found that mono-alpha substitution in the side chain had little effect upon

activity. The α,α -disubstituted compounds (i.e. aryl-OCC(CH₃)₂COOH) were inactive in the straight growth test and showed but slight activity in the pea test. Due to the inactivity of the α,α -disubstituted derivatives, Wain and coworkers proposed the hypothesis that there is a chemical reaction involving a hydrogen atom in the alpha position, and that at least one such hydrogen atom is necessary for activity.

The activity of α -methylenephylacetic acid and the high activity of 2,3,6-trichlorobenzoic acid would indicate that this alpha hypothesis is invalid. As mentioned previously, the inactivity of the α,α -disubstituted acids could be due conceivably to steric hindrance, which prevents the molecule from fitting into a requisite pattern necessary for activity.

As previously mentioned, Muir and Hansch (71) proposed a two-point reaction theory. This consisted of an amide linkage with the carboxyl group and a nucleophilic attack on an ortho position by a sulfhydryl group. The reactive substrate unit being a free cysteine group. This two-point theory has considerable evidence in its favor. Foster, McRae and Bonner (33) have shown by a study of enzyme kinetics that inhibition of growth by growth-regulators at higher concentrations would be expected if the substance reacts by a two-point attachment mechanism. The active complex would have a growth-regulator molecule attached at two points on the substrate; as the concentration of regulator increases, the probability of two molecules attaching at the two substrate sites increases and the number of two-point attachments of one growth-regulator molecule forming the active complex progressively diminishes.

Thimann and Bonner (104,105) observed that iodoacetate, arsenate and

p-chloromercuribenzoate (which react with sulfhydryl groups) inhibit the elongation of Avena sections. This may be interpreted as indicating some type of reaction between sulfhydryl enzymes and the growth-regulator.

Bonner (17) also reported an inhibition in the growth of Avena coleoptile sections by L-cysteine, indicating a preferential reaction. The fact that cysteine is used for detoxification of bromo-, chloro- and iodobenzene and of naphthalene in animals would indicate that the hypothesis for formation of the thio ether is not unreasonable. Bromobenzene is converted to p-bromophenyl-mercapturic acid (90) and naphthalene to α -naphthylmercapturic acid (19,53). In both cases, then the amino group has been acetylated; this further supports the two-point attachment theory.

As Muir and Hansch (71) point out, this hypothesis explains very satisfactorily the activity of cis-cinnamic acid in elongation and the inactivity of the trans form as well as other cis-trans acids of this type, since the two-point attachment results in a ring structure. The ease of ring formation in a cis-acid over that of the trans-isomer is obvious.

The interpretations of the two-point attachment theory do not account for all of the observed results and the proposers have not attempted to explain the extreme specificity of certain substituents and positions. The author cannot agree with the proposal of a nucleophilic attack on the benzene ring. Nucleophilic displacements of aromatic hydrogen do not ordinarily occur. Benzene itself is fundamentally nucleophilic and the final ejection of a hydrogen anion is unlikely. Nucleophilic substitution at an aromatic carbon should be facilitated by: (a) any influence which increases the normally unreactive character of the aromatic nucleus, and (b) by substituting

for the hydrogen which is displaced as a negative ion, some atom or group which more readily exists as a negative ion. In other words, a powerful meta orienting group should facilitate the SN_2 (bimolecular nucleophilic substitution) type displacement of any group capable of existing as an anion when this second group is located ortho or para to the meta orienting group. The following examples are illustrative. The hydrolysis of chlorobenzene to phenol is only accomplished at elevated temperatures and pressures; the Dow Chemical Co. prepares phenol commercially in this manner (38) and uses 6-8% aqueous NaOH at 300° C and 2000-3000 p.s.i. pressure. In distinct contrast, o- and p-nitrochlorobenzenes are readily hydrolyzed by aqueous Na_2CO_3 at 130° C and atmospheric pressure (124). This labelizing effect, as would be expected, is not observed with m-nitrochlorobenzene. Thus, a nucleophilic displacement of a hydrogen atom ortho or para to a strong ortho-para directing group (such as the carboxymethoxy group) is very improbable if not impossible. The mechanism proposed for the substituted benzoic acids, as will be seen later, follows the prerequisites for aromatic nucleophilic displacement.

If the ring closure at the ortho position is not accomplished by an attack of the nucleophilic SH group, it seems logical to postulate the intermediate formation of a sulfonium or carbonium ion (the only evidence of S-C bond formation is the detoxification reaction in animals) and an electrophilic attack. From an electronic view point, the carbonium ion formation would be the logical choice, giving rise to a reaction similar to a Friedel-Craft reaction. This hypothesis would meet all of the requirements for attachment to a position ortho to the carboxymethoxy group. There are three

factors which seem to be of an obvious importance in determining an active structure: (a) the position of the aromatic substituent, (b) the electronic effect of this substituent relative to further substitution and (c) the size of the substituent. Factors (a) and (b) are closely tied together, i.e. before a substituent can exert an influence, it must be located in a suitable position. The size factor would explain why only the halogens and the methyl group are particularly effective substituents. A comparison of atomic radii shows the methyl group to be almost the same size as the iodine atom.

Although the hydroxy group and the amino group have a suitable size, they would be excluded by factors (a) and (b). Both of these groups have a stronger +T effect than the OCH_2COOH group and would, when located ortho or para to the side chain, cause substitution ortho or para to themselves (upon construction of Hirschfelder molecular models, it can be seen that the only positions capable of forming a ring with the cysteine residue are the positions ortho to the side chain. The meta positions will form ring structures, but only with considerable strain). If the OH or NH_2 group is located in the meta position of phenoxyacetic acid, it would be para to the 2 position. The 2 and 4 positions are then most prone to electrophilic attack, probably in the order of 2 greater than 4. This reasoning would predict meta-hydroxyphenoxyacetic acid to be highly active, but experimental evidence shows it to be inactive. However, it must be remembered that the attacking cysteine unit is most probably attached to a protein residue through its carboxyl group and attack by such a bulky molecule would be hindered to a much greater extent by the OCH_2COOH group as compared to the

relatively small hydroxy or amino group. The importance of size is evident in the substitution of alkyl benzenes (65). As the alkyl group of the alkyl benzene increases in size from methyl to tert-butyl, para substitution becomes increasingly favored. Similarly, as the size of the attacking reagent becomes larger, para substitution should be favored. This hindrance of and by the attacking molecule may well explain why the postulated factor (c) is of a paramount importance. If the +T effect of a meta substituent is only slightly greater than that of the OCH_2COOH group and the size greater than that of the OH or NH_2 , some activity would be expected. The methoxy group fits both requirements and has been reported to be active when in the meta position (119).

When a substituent is strongly electron attracting (by permanent polarization) the near-by ortho positions should be more strongly deactivated than the more distant para position and the ortho-para ratio should decrease (3). This electronic effect is apparently more important than slight size increases, since the nitration of the halobenzene series the size of the substituent increases from fluorobenzene to iodobenzene, but ortho nitration becomes increasingly more pronounced in descending the series. It is well known that the electron-attracting power of the halogens also decreases in the same order. This could explain why phenylacetic acid is highly active and phenoxyacetic acid is virtually inactive. Two compounds which would be analogous in their substitution reactions are phenol (to phenoxyacetic acid) and toluene (to phenylacetic acid). With an entering substituent, phenol directs predominantly to the para position, while toluene may have an ortho-para ratio greater than 1:0 (50). Thus, if

phenoxyacetic acid is preferentially attacked in the para position, it could act at best as an antiauxin. Either para substitution takes place and no ring closure by the amide linkage or preferably, the amide linkage (or the intermediate salt linkage) is formed and no ring closure follows due to the directed para substitution.

The differences in the activity of the three chlorophenoxyacetic acids could be accounted for by the above considerations. The chlorine atom is ortho-para directing, but due to the relatively high -I effect, the ring is less easily substituted than benzene. The relative rates of nitration (benzene = 1.0) of chlorobenzene in the ortho and para positions are 0.030 and 0.139 respectively. So the predicted order of activity would be para > meta > ortho. This is the observed order obtained by many investigators. The effect of the ortho chlorine atom on further substitution may explain why o-chlorophenoxyacetic acid is active (the activity is low but significant) and phenoxyacetic acid is inactive. The acetylamino group has nearly the same electronic effects as the OCH_2COOH group and the following data would apply with only minor differences to phenoxyacetic and o-chlorophenoxyacetic acid: (a) acetanilide yields on nitration 96% para substitution (49), (b) ortho-chloroacetanilide on nitration yields only 59% para substitution (27), the increase in ortho substitution being nearly 10 fold. It is interesting to note that in the substitution of the three chloroacetanilide isomers, the amount of ortho substitution increases in the order of para > meta > ortho (26). Another factor which probably aids the ortho substitution of o-chlorophenoxyacetic acid would be the resultant non-coplanarity of the OCH_2COOH group. This partial restriction of rotation may

decrease the hindrance encountered by the electrophilic agent, and must in turn be of greater importance than the effect on resonance due to non-coplanarity.

The presence of a meta directing group in the benzene ring greatly inhibits electrophilic substitution, especially when the attacking group is large. The attack by the postulated plant substrate is comparable to alkylation by the Friedel-Crafts procedure. In both cases the size of the attacking group is relatively large and steric factors become of paramount importance. One characteristic feature of alkylation by the Friedel-Crafts procedure is that meta directing groups, with few exceptions, prevent reaction completely. This could then explain the inactivity of all compounds which contain a meta-directing group.

The effect of two or more substituents in phenoxyacetic acid would be difficult to predict without supporting experimental evidence. However, the high activity of 2,4-D and the inactivity of 2, 6-dichlorophenoxyacetic acid are obvious from the above considerations. The activity of 3,4-dichlorophenoxyacetic acid can be accounted for by the additive effects of a para and meta chlorine. 2,3- and 3,5-dichlorophenoxyacetic acid would direct primarily to the para position and due to multiple chlorine substitution, may have little direction to the ortho position. The high activity of 2, 5-dichlorophenoxyacetic acid would be difficult to explain, unless for some reason it directs primarily to the ortho position.

There are many other compounds, whose activity or inactivity can be well explained by use of electronic theory, but the author mainly wishes to point out the strong possibility that the two-point attachment is in part

an electrophilic attack, the probability of occurrence being determined by the electron density at the ortho position (for compounds as the phenylacetic and phenoxyacetic acids). Thimann (102) was the first to suggest some correlation between activity and aromatic orientation, but he failed to give any supporting evidence. The only serious objection to this hypothesis is the evidence reported by Wain (116) that 2,4-dichloro-6-fluoro- and 2,4-dibromo-6-fluorophenoxyacetic acids have high activity in the elongation of *Avena coleoptile* sections.

The Benzoic Acids

Substituted benzoic acids were introduced as possible growth-regulators by Zimmerman and Hitchcock (60). They reported a slight activity for 2-bromo-3-nitrobenzoic acid in cell elongation and formative effects with 2-chloro-5-nitro- and 2,3,5-triiodobenzoic acid. Bentley (13) reported a high activity for 2,3,6-trichlorobenzoic acid in the elongation of *Avena coleoptile* sections. This finding which was corroborated in other tests (102, 112, 132), stimulated a renewed interest in compounds of this type and led to the investigation of many other substituted benzoic acids (72, 111, 133, 69).

From the results obtained by these investigators it can be concluded that substituents having a strong +T effect do not confer activity while those substituents with relatively weak ortho para directing influence (Cl, Br, I, CH₃, etc.) may do so. The nitro group may or may not be effective in the 3-position. Ortho-substitution "activates" benzoic acid, and appears to be essential. Di-ortho substitution causes a more pronounced activity and 2,3,6-derivatives seem to give a maximum response. Indications are that the 4-position must remain free.

Due to the apparent necessity of ortho substitution, Muir and Hansch (72) suggested that the activity of these compounds involved the displacement of an electron-attracting group (halogen or nitro) in the ortho position, and that there is a two-point attachment with a cysteine unit in the same manner as that proposed for the phenoxyacetic acids. Hansch et.al. (40) were able to demonstrate the release of chloride from 2,6-dichlorobenzoic acid during the growth of *Avena* coleoptile sections, thus substantiating this hypothesis. According to Veldstra and van de Westeringh (110) the data of Hansch et.al. do not permit the conclusion that the release of chloride ion is essentially connected with the physiological activity of the compounds; they point out that the inactive 2,4-dichlorobenzoic acid releases chloride ion and such a release should not be found for an inactive compound. Veldstra (110,113) further points out that on the basis of the ortho reaction concept, 2,6-dibromobenzoic acid should be active, but it was found to be inactive (110). On the other hand, 2,6-dimethylbenzoic acid is slightly active, 3-nitro-2,6-dimethylbenzoic acid is active and 3-chloro-, 3-bromo-, and 3-iodo-2,6-dimethylbenzoic acid are highly active (110). Because of this Veldstra concludes that a reaction with a plant substrate involving displacement of the methyl group or another form of chemical reaction with the inert ortho substituent can be ruled out.

This author is inclined to agree with Veldstra in that the above and following evidence tends to invalidate the ortho reaction hypothesis. As was pointed out in the discussion of the phenoxyacetic acids, the aromatic ring containing a meta directing group located ortho or para to a group which can readily exist as an anion, is prone to nucleophilic attack. The

mechanism proposed by Muir and Hansch (72) is a nucleophilic displacement ortho to the carboxyl group. Although this mechanism would explain the activity of certain ortho substituted benzoic acids, it cannot account for the inactivity of such compounds as 2-chloro-5-nitrobenzoic acid and 2,4-dichlorobenzoic acid. If the activity is dependent on a nucleophilic displacement in the ortho position, 2-chloro-5-nitrobenzoic acid, with the nitro group para to the ortho chloro group, should have a very high activity and it is totally inactive (72). Holleman and De Mooy (51) list the following order for relative rate constants for a number of dichloronitrobenzenes in their nucleophilic replacement reaction with sodium methoxide: 2,4; 3,4; 2,5; 2,3; 2,6 (the relative rate constants in hours⁻¹ being 19.41; 17.42; 3.93; 1.74 and 0.14 respectively). All these compounds have an ortho chloro group except the 3,4-isomer and it is seen that the least reactive isomer is the 2,6-derivative; this low reactivity is explainable by the steric inhibition of resonance, i.e. any structural modification which tends to destroy coplanarity inhibits resonance and without resonance the nitro or carboxyl group cannot exert its -T effect. As was state above, the 2,6-disubstituted benzoic acids are among the most active as plant growth regulators, so it would seem that if a nucleophilic mechanism is operating, some factor of a paramount importance is giving rise to the discrepancy in the results which have been obtained thus far.

The author does not like to rule out the nucleophilic displacement hypothesis as there is as much evidence in its favor as against it. Only through extensive study of further derivatives can the problem be clarified.

Activity and Optical Isomerism

Kogl and Verkaaik (59,60) found (+) α -indole-3-propionic acid to be 30 times as active in the Avena curvature test as the (-) isomer. In the straight growth test the two isomers were equally active. However, it was proved that the differences in the Avena curvature test were caused by retention of the (-) acid in the apex by selective adsorption.

Later, however, differences in activity between optical enantiomorphs were found in the straight growth and pea tests (88,89,109,115,101,1). A survey of these results shows that the (+) form showed the strongest growth substance action. Fredga and Matell (34) and Matell (68) established that the active enantiomorph of a dl pair has the d configuration. Veldstra (113) states that these results stress the eminent importance of the spatial form of the active molecule, and if the growth response depends on the degree of fitting of the active molecule on the primary active site, one would expect the most active antipodes to belong to the same steric series, irrespective of their sign of rotation. It must be remembered, however, that the most active growth substances, indoleacetic acid, naphthalene-acetic acid and 2,4-dichlorophenoxyacetic acid are not optically active and that introduction of an alpha substituent must give rise to some steric hindrance with the interaction of the growth substance and its cellular substrate.

Non-acidic Compounds

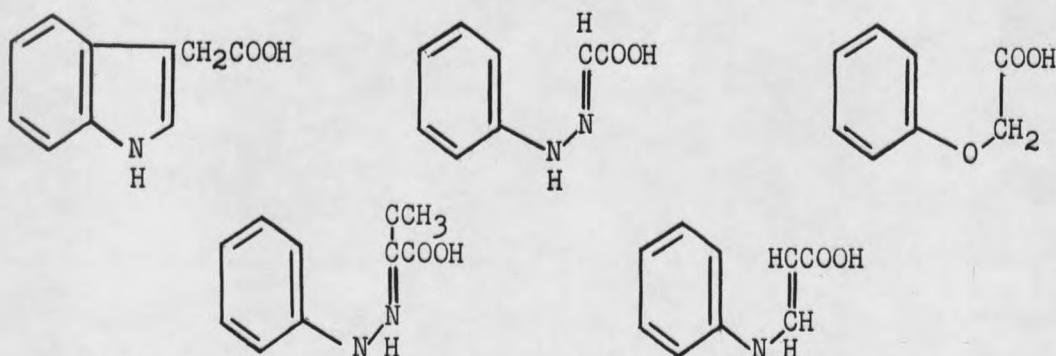
Acid derivatives, such as esters and amides have been found generally active, but to a lesser extent than the active free acid. It is generally accepted that a conversion of the derivative into the parent acid is a pre-

requisite for activity.

All indications are that the activity of aldehydes (113,5,63), nitriles (101,127) and alcohols (113) is due to a conversion to the corresponding acid. There is some indication, however, that some nitriles are active per se (42,54).

New Plant Growth-regulators

The two main series of compounds which were chosen for study were the substituted phenylhydrazones of glyoxylic and pyruvic acids. Several β -anilinoacrylic acids were also synthesized and tested. The structural similarity of these compounds to indoleacetic acid and the phenoxyacetic acids is obvious:



To the author's knowledge, the only compounds of a similar structure which have been tested as plant growth-regulators are the mono- and di-nitro-phenylhydrazone derivatives of levulinic acid (70) and β -diphenylamino-propionic acid (4). All these compounds were reported to show activity as growth-regulators or herbicides. The phenylhydrazono group is similar in size to the phenoxy group and has an ortho-para directing influence on the benzene ring. It should not be unlikely, then for these compounds to show activity as plant growth-regulators. The same arguments would apply to the

β -anilinoacrylic acids.

2-nitro-4-methoxyphenoxyacetic acid was also synthesized and tested in the hope that the combined orientation effects of the nitro (strong -T) and the methoxy (strong+T) groups would parallel that of the 2,4-dichloro derivative.

α -Phenyliminopropionic acid was also prepared and tested.

IV. EXPERIMENTAL

The test plants were grown according to Bonner (15). The headrow oat seeds were germinated in sand in the dark at a temperature of 29° C and a relative humidity of 80-90%. After 96 hours the apical 5 mm (approx.) of the coleoptile were removed and two hours later sections were cut from the apex with the primary leaf included within the coleoptile. No more than two sections were cut from one coleoptile. The cutter used gave sections 7.61 units (5.74 mm) in length with a standard deviation of 0.03 unit. Single determinations containing either 10 or 15 sections were run. The sections were floated on the surface of 25 ml of the solution in a Petri dish and placed in an incubator at 28° C for 24 hours. Measurements were made with an ocular micrometer mounted in a binocular microscope (13X).

Before planting, the sand was leached with dilute hydrochloric acid and then with distilled water until the filtrate proved free from chloride ion.

Preparation of Solutions

Whenever possible, the compounds were simply dissolved in distilled water. The addition of base was necessary in many instances, due to the low solubility of the free acid. In all cases, however, the pH of the solution was below 7.0. Due to the very low solubility of the esters, an emulsion was employed. The following example of ethyl α -phenylhydrazonopropionate is typical: 0.2062 g of ethyl α -phenylhydrazonopropionate was placed in a 250 ml volumetric flask and 1 ml of EMCOL H-68c (generously supplied by the Emulsol Chemical Corporation) solution (10% in xylene) was added and the mixture gently shaken until complete solution was obtained.

Approximately 150-200 cc of water were added and the mixture shaken vigorously to yield a milky-white emulsion. The emulsion was then made up to volume and shaken. This procedure yields a 0.004 M emulsion of the ester and contains ca. 0.4% xylene and ca. 0.04% EMCOL H-86c. This concentration of xylene and emulsifier and the concentrations contained in subsequent dilutions were shown to have no effect on the growth of the coleoptile sections.

α -Phenylhydrazonopropionic Acid

8.8 g (0.1 mole) of pyruvic acid were dissolved in 40 cc of ethyl ether and 10.8 g (0.1 mole) of phenylhydrazine dissolved in 50 cc of ethyl ether were added with stirring during a period of 5 minutes. The light yellow precipitate which formed immediately, was removed by filtration, washed twice with ether and air dried. The hydrazone was recrystallized from dilute alcohol to yield light yellow needles. The yield was 16.5 g (97% of theoretical). Corrected melting point: 192° C. Curtuis (26) gives the melting point as 192° C.

Phenylhydrazonoacetic Acid (21)

20 g of dichloroacetic acid were dissolved in 20 cc of water and a solution of 34 g of potassium hydroxide in 60 cc of water was added. To this solution, 16 g of phenylhydrazine dissolved in 20 cc of 95% ethyl alcohol were added and the resulting solution refluxed for two hours. The alcohol was then removed by distillation and the aqueous solution extracted with three 20 cc portions of ether. The remaining ether was removed by distillation. The solution was allowed to cool to room temperature (ca. 25° C) and strongly acidified with acetic acid. The red-orange precipitate

was filtered off and dissolved in ca. 2500 cc of warm water (45-50° C, a temperature higher than this gives rise to extensive decomposition upon the following acidification). The solution was filtered and 30 cc of concentrated hydrochloric acid was added. Upon cooling to 5-10° C, long dark brown needles separated. Purification was accomplished by redissolving the product in 2500 cc of warm water (45-50° C) containing 10 g of potassium hydroxide, stirring with a small amount of Norite, filtering and acidifying with 30 cc of concentrated hydrochloric acid. Cooling of the solution yielded large light brown needles. The yield was 19.6 g (81% of theoretical). Melting point with decomposition: 141° C (corrected). Muller (75) gives the melting point as 142-3° C.

Busch et.al. (21) obtained two isomeric forms with this procedure, but the author was unable to duplicate their results on many attempted runs.

Due to the instability of this compound, it was converted to its potassium salt by treating its alcoholic solution with the equivalent amount of alcoholic potassium hydroxide. The precipitated salt was washed with absolute alcohol and dried in a vacuum desiccator. Analysis of percent potassium: calculated, 19.34%; found, 19.28%.

α -Phenyliminopropionic Acid

8.8 g (0.1 mole) of pyruvic acid were dissolved in 40 cc of ethyl ether and a solution of 9.3 g (0.1 mole) of aniline dissolved in 40 cc of ether was added with stirring. The light yellow precipitate was filtered off and recrystallized from an alcohol-benzene solution. The yield was 6.8 g (42% of theoretical). Melting point: 127-28° C (corrected). Beilstein (8) lists the melting point as 127-28° C.

Ethylformylacetate, Sodium Enolate (125)

In a three-necked flask fitted with a reflux condenser, a mercury-seal stirrer, and a drop funnel were placed 350 cc of anhydrous ether and 23 g of sodium wire. A mixture of 90 g of ethyl acetate and 85 g of ethyl formate was slowly added over a period of approximately one hour. The mixture was stirred under gentle reflux for approximately 4 hours. 5 cc of 95% ethyl alcohol were then added and the mixture refluxed an additional 4 hours, or until the last traces of sodium had disappeared. Upon standing a dense yellow precipitate formed. The precipitate was removed by suction filtration and allowed to air dry. The precipitate consisted of fine yellow needles. The yield was 86 g. By repeated washing with dry ether a nearly colorless product was obtained. Analysis showed the compound to be 75.5% pure; this compares to the results obtained by Wislicenus (125).

Ethyl β -anilinoacrylate (82)

4.6 g (0.05 mole) of aniline was dissolved in 100 cc of 5% acetic acid. A solution of ethylformylacetate, sodium enolate (9.6 g in 50 cc of water; equivalent to 0.05 mole of enolate) was added with stirring. The yellow precipitate which formed immediately, was filtered off and recrystallized from 95% alcohol. The product separated as light yellow plates. The yield was 5.4 g (57% of theoretical). Melting point: 113° C. Straus and Voss (94) give the melting point as 114-14.5° C.

2,4-Dichloroacetanilide (22,86)

100 g of acetanilide were dissolved in 800 cc of glacial acetic acid and 125 g of sodium acetate (freshly fused and powdered) were added. The flask and its contents were tared and chlorine was passed into the mixture

through a special addition tube (30). This addition tube was necessary to prevent clogging by the precipitation of p-chloroacetanilide and later the 2,4-dichloroacetanilide. As soon as the p-chloroacetanilide had completely precipitated (absorption of ca. 53 g of chlorine), the flask was heated on a water bath and the passage of chlorine was continued until a total of 105 g had been absorbed. The mixture was allowed to cool and the solid which separated was removed by filtration. The sodium chloride was removed from the precipitate by digestion with water. No attempt was made to recover all of the 2,4-dichloroacetanilide, since the mother liquor was used for the chlorination of another 100 g of acetanilide (125 g of sodium acetate again being added). After the solid from the second chlorination had been removed by filtration, the mother liquor was diluted with an equal volume of warm water and the mixture cooled to ca. 5° C. The solid which precipitated was removed and combined with the first two yields. The combined product was digested with water to insure complete removal of sodium chloride, dried and recrystallized from 95% ethyl alcohol. The recrystallization yielded large white crystals which gave a corrected melting point of 145° C. Chattaway et.al. (22) report a melting point of 144° C. The total yield was 268 g (89% of theoretical).

2,4-Dichloroaniline (22)

268 g of 2,4-dichloroacetanilide were dissolved in 450 cc of concentrated sulfuric acid and the solution heated at a temperature between 110 and 120° C for ca. one hour. The solution was allowed to cool to room temperature (ca. 26° C) and poured slowly into 2 liters of cold (0° C) 25% sodium carbonate solution. The heavy white precipitate was filtered off

and washed once with ice cold water. The 2,4-dichloroaniline was then suspended in ca. 2 liters of cold water and 40% NaOH was added until the mixture was basic to litmus. After stirring rapidly for 30 minutes, the 2,4-dichloroaniline was filtered off and air dried. The yield was 202 g (94.8% of theoretical). Melting point: 62-63° C. Hyde (52) gives the melting point as 62.5-63° C. If the 2,4-dichloroacetanilide is of high purity, no further purification of the resulting aniline is necessary.

2,4-Dichlorophenylhydrazine Hydrochloride

With several modifications, the procedure of Coleman (25) for the preparation of phenylhydrazine was followed. In a 500 ml, 3-necked flask, fitted with a mechanical stirrer, were placed 62.5 cc of concentrated commercial hydrochloric acid (22 Bé). 40.5 g (0.25 mole) of 2,4-dichloroaniline were then added and the mixture stirred until a thick, homogenous paste was formed. The flask was surrounded with a freezing mixture of ice and salt, and when the contents were 0° or lower, stirring was started and 62 g of cracked ice were added; then a cold solution (0° or lower) of 18.1 g of NaNO₃ dissolved in 38 cc of water was allowed to run in slowly (20-30 minutes) from a dropping funnel, the end of which was drawn to a small tip and reached nearly to the bottom of the flask. Vigorous stirring was maintained and the temperature was kept as near zero as possible by frequent addition of cracked ice (up to 65 g).

During this addition, a sodium sulfite solution was prepared by dissolving 80 g of anhydrous sodium sulfite in 375 cc of water. The solution was placed in a 3 liter, 3-necked flask and cooled to minus 5° C. Approximately 30 g of ice was added to this solution and with rapid stirring the

diazonium salt solution was added as rapidly as possible. If the two solutions are not sufficiently cold, poor reduction results due to loss of SO_2 . The flask was then warmed on a steam bath to 70°C , and 25 cc of concentrated hydrochloric acid was added (testing with litmus to be sure the solution was acid). The flask was kept at $60-70^\circ \text{C}$ for ca. 2 hours.

250 cc of concentrated commercial hydrochloric acid were then added and the mixture was cooled to 0° . The precipitated 2,4-dichlorophenylhydrazine hydrochloride was filtered off and air dried. The product was recrystallized from a dilute ethyl alcohol solution to which a few cc of concentrated hydrochloric acid had been added. The compound was dried overnight in a vacuum desiccator. The yield was 44.1 g (82.5% of theoretical).

Analysis. Calculated for $\text{C}_6\text{H}_7\text{N}_2\text{Cl}_3$: Cl, 49.83. Found: Cl, 49.69.

Neutralization equivalent. Calculated: 213.5; found: 212.8.

The melting point was 212°C with decomposition. Chattaway and Pearce (23) give the melting point as 210°C with decomposition. The free base is obtained by treating an aqueous solution of the hydrochloride with excess base. Recrystallization from dilute alcohol yields needles; melting point was 94°C . Chattaway and Pearce give 94°C .

The procedure given for the preparation of 2,4-dichlorophenylhydrazine hydrochloride is a general one and was used with only minor changes for the preparation of the following arylhydrazine hydrochlorides.

p-Tolylhydrazine Hydrochloride

The product was recrystallized from ca. 2 N. hydrochloric acid and separated as colorless needles. The yield was 105 g (86% of theoretical). Treatment of the hydrochloride with excess base liberated the hydrazine,

which separated as colorless leaflets upon recrystallization from dilute alcohol. Melting point of the free base: 64°C . Bamberger (7) gives the melting point at 65°C .

p-Chlorophenylhydrazine Hydrochloride.

The compound was recrystallized from water which contained a few cc of concentrated hydrochloric acid. It crystallized as a fine powder and melted with decomposition at $228-230^{\circ}\text{C}$. The yield was 46 g (52% of theoretical). Beilstein (10) gives the melting point as $225-230^{\circ}\text{C}$ with decomposition.

Analysis. Calculated for $\text{C}_6\text{H}_8\text{N}_2\text{Cl}_2$: Cl, 39.67. Found: Cl, 39.56.

Neutralization equivalent. Calculated: 179.0; found: 178.2

The free base crystallized as needles from ether; melting point 90°C . Beilstein (10) gives 90°C .

o-Chlorophenylhydrazine Hydrochloride

The compound crystallized from dilute alcohol as fine yellow needles. The yield was 56 g (63% of theoretical). Melting point: 194°C . with decomposition. Beilstein (9) lists the melting point as 194°C with decomposition.

Analysis. Calculated for $\text{C}_6\text{H}_8\text{N}_2\text{Cl}_2$; Cl, 39.67. Found: 177.9

The free base crystallizes as needles from dilute alcohol. Melting point: 47°C . Beilstein (9) gives $46-47^{\circ}\text{C}$.

2,5-Dichlorophenylhydrazine Hydrochloride

The hydrochloride separated as very fine light brown needles from dilute alcohol. Yield was 49.6 g (93% of theoretical).

Analysis. Calculated for $\text{C}_6\text{H}_7\text{N}_2\text{Cl}_3$; Cl, 49.83; N, 13.12. Found:

Cl, 49; N, 13.06.

Neutralization equivalent. Calculated: 213.5; found: 212.6.

The free base crystallizes as needles from water. Melting point: 106°C. Zettel (126) gives the melting point as 105° C.

α -(p-Tolylhydrazono)-propionic acid

15.86 g (0.1 mole) of p-tolylhydrazine hydrochloride were dissolved in 80 cc of water (ca. 70° C) and 8.9 g (0.1 mole) of pyruvic acid dissolved in 20 cc of water were added with stirring. The yellow precipitate which formed was filtered off after the mixture had cooled to ca. 10° C. The hydrazone was recrystallized from dilute alcohol. It crystallized as yellow needles and melted at 161°C. The yield was 7.4 g (39% of theoretical). Beilstein (11) gives the melting point as 162°C.

Neutralization equivalent. Calculated for $C_{10}H_{12}N_2O_2$; 192.2; found: 192.8.

Ethyl β -(p-methylanilino)-acrylate

The procedure used was similar to the one employed in the preparation of ethyl β -anilinoacrylate. The yellow precipitate which formed was recrystallized from dilute alcohol to yield light yellow plates. The yield was 4.1 g (40% of theoretical). Melting point: 116° C. Pechmann (82) gives the melting point as 116° C.

Analysis. Calculated for $C_{12}H_{15}NO_2$: N, 6.82. Found: N, 6.73.

α -(o-Chlorophenylhydrazono)-propionic Acid

8.95 g (0.05 mole) of o-chlorophenylhydrazine hydrochloride were dissolved in 250 cc of warm water (70-80° C) and 4.45 g (0.05 mole) of pyruvic acid dissolved in 10 cc of water were added with shaking. A yellow

precipitate formed immediately and after cooling the mixture to ca. 20° C, the precipitate was filtered off and recrystallized from alcohol. The yield was 3.5 g (33% of theoretical). The hydrazone crystallized as lemon-yellow needles. Melting point: 176° C. Hewitt (44) gives the melting point as 178° C.

Analysis. Calculated for $C_9H_9N_2O_2Cl$: Cl, 16.68. Found: Cl, 16.73.

Neutralization equivalent. Calculated: 212.6; found: 212.6.

α -(p-Chlorophenylhydrazone)-propionic Acid

The procedure was the same as that used for α -(o-chlorophenylhydrazone)-propionic acid. The hydrazone was recrystallized from dilute alcohol and separated as lemon-yellow needles. The yield was 5.3 g (50% of theoretical). Melting point: 199° C. Hewitt (43) gives the melting point as 199° C.

Analysis. Calculated for $C_9H_9N_2O_2Cl$: Cl, 16.68. Found: Cl, 16.61.

Neutralization equivalent. Calculated: 212.6; found: 213.4.

Ethyl 2,4-Dichlorophenylhydrazonoacetate (24)

10.68 g (0.05 mole) of 2,4-dichlorophenylhydrazine hydrochloride, 8.27 g (0.05 mole) of chloral hydrate, and 50 cc of absolute alcohol were placed in a 200 ml round-bottom flask which was provided with a reflux condenser. The mixture was slowly heated on a steam bath until evidence of reaction occurred (ca. 65° C). The mixture was then slowly heated to boiling and gently refluxed for 30 minutes. Approximately 25 cc of alcohol were removed by distillation and the residue was allowed to cool to room temperature. The brownish-yellow prisms which precipitated were filtered off and recrystallized from 95% alcohol. The recrystallization yielded pale yellow prisms. The yield was 6.9 g (53% of theoretical). Melting

point: 122° C. Chattaway and Bennett (24) give the melting point as 121.5° C.

Analysis. Calculated for $C_{10}H_{10}N_2O_2Cl_2$: Cl, 27.16. Found: Cl, 27.09.

2,4-Dichlorophenylhydrazonoacetic Acid

5 g (0.019 mole) of ethyl 2,4-dichlorophenylhydrazonoacetate were added to a flask containing a 10% potassium hydroxide solution in 95% alcohol. The solution was refluxed for ca. 1.5 hours and 70 cc of water were added. The alcohol was removed by distillation and the clear red residue was cooled to room temperature. 20 cc of concentrated hydrochloric acid were added with stirring and the resultant greenish precipitate removed by filtration. All attempted recrystallizations resulted in a finely powdered product. The yield was 3.8 g (85% of theoretical). Melting point was $158-9^{\circ}$ C. No literature value was found.

Analysis. Calculated for $C_8H_6N_2O_2Cl_2$: Cl, 30.42. Found: Cl, 30.50.

Neutralization equivalent. Calculated: 233.1; found: 233.1.

Ethyl α -Chlorophenylhydrazonoacetate

8.95 g (0.05 mole) of p-chlorophenylhydrazine hydrochloride, 8.3 g (0.05 mole) of chloral hydrate and 100 ml of absolute alcohol were placed in a 200 ml round-bottom flask and slowly heated to boiling. The solution was refluxed for 30 minutes and after cooling was poured into ca. 500 cc of cold water. The brownish precipitate was filtered off and recrystallized from dilute alcohol. The yield was 7.4 g (65% of theoretical) of fine needles. Melting point: 132° C with decomposition. No literature value was found.

Analysis. Calculated for $C_{10}H_{10}N_2O_2Cl$: Cl, 15.64. Found: Cl, 15.61.

Ethyl 2, 5-Dichlorophenylhydrazonoacetate

The procedure was similar to that used for the preparation of ethyl 2,4-dichlorophenylhydrazonoacetate. Recrystallization from dilute alcohol yielded a fine yellow powder (which consisted of microscopic prisms). The yield was 7.1 g (54% of theoretical). Melting point: 85-6° C. No literature value was found.

Analysis. Calculated for $C_{10}H_{10}N_2O_2Cl_2$: Cl, 27.16. Found: Cl, 27.22.

α -(2,4-Dichlorophenylhydrazono)-propionic Acid

10.6 g (0.05 mole) of 2,4-dichlorophenylhydrazine hydrochloride were dissolved in 100 cc of water (ca. 80-90° C) and a solution of 4.4 g (0.05 mole) of pyruvic acid dissolved in 40 cc of water was added with stirring. The stirring was continued for 15-20 minutes; the mixture was cooled and the precipitate filtered off. Recrystallization from dilute alcohol yielded fine yellow crystals. The yield was 4.8 g (39% of theoretical). Melting point: 198-99° C. No literature value was found.

Analysis. Calculated for $C_9H_8N_2O_2Cl_2$: Cl, 28.70. Found: Cl, 28.61.

Neutralization equivalent. Calculated: 247.1; found: 247.9.

Ethyl β -(p-chloroanilino)-acrylate

The procedure was the same as that used for the preparation of ethyl β -anilinoacrylate, except hydrochloric acid was used in place of acetic acid. The oil which formed upon addition of the enolate solidified on cooling. The solid was recrystallized from alcohol to give a brown powder. The yield was 4.1 g (36% of theoretical). Melting point: 67-70° C (no sharp range). No literature value was found.

Analysis. Calculated for $C_{10}H_{12}NO_2Cl$: Cl, 16.76. Found: Cl, 16.68.

Ethyl α -Phenylhydrazonopropionate

26.7 g (0.15 mole) of α -phenylhydrazonopropionic acid, 150 cc of absolute alcohol and 5 g of concentrated sulfuric acid were placed in a flask and refluxed for 4.5 hours. The solution was cooled and poured into 1000 cc of cold water. The oil which formed solidified with stirring. The solid was filtered off and recrystallized from dilute alcohol. The ester separated as large yellow prisms. The yield was 24.3 g (79% of theoretical). Melting point: $192^{\circ}C$. Fisher (32) gives the melting point as $192^{\circ}C$.

α -(p-Bromophenylhydrazono)-propionic Acid

11.2 g (0.05 mole) of p-bromophenylhydrazine hydrochloride were dissolved in 50 cc of dilute alcohol. To this solution was added a solution of 4.4 g (0.05 mole) of pyruvic acid dissolved in 50 cc of water. The mixture was heated to boiling and refluxed for 10 minutes. On cooling, long light brown needles separated. The product was recrystallized from dilute alcohol to yield 7.3 g (57% of theoretical) of light yellow-brown needles. Melting point: $184^{\circ}C$. Balbiano (6) gives the melting point as $182^{\circ}C$.

Neutralization equivalent. Calculated for $C_9H_9N_2O_2Br$: 257.1; found: 257.2.

α -(p-Nitrophenylhydrazono)-propionic Acid

7.6 g (0.05 mole) of p-nitrophenylhydrazine and 4.5 cc of concentrated hydrochloric acid were placed in a flask and 50 cc of water were added. The mixture was heated until solution was complete. To this solution a solution of 4.4 g (0.05 mole) of pyruvic acid dissolved in 50 cc of water was added with shaking. The mixture solidified to a gel and the solid was

filtered off and recrystallized from dilute alcohol. The hydrazone separated as a yellow powder (composed of microscopic needles). The yield was 10.2 g (91% of theoretical). Melting point: 219°C . Hyde (52) gives the melting point as $219-20^{\circ}\text{C}$.

Neutralization equivalent. Calculated: 223.2; found: 223.9.

2-Nitro-1,4-dimethoxybenzene (37)

41 g of 1,4-dimethoxybenzene were added to 500 cc of water and the mixture was heated with stirring until the ether had melted (ca. 50°C). added and the temperature was held between $50-55^{\circ}\text{C}$. The yellow oil which formed gradually solidified and after the mixture had cooled to room temperature, the yellow solid was filtered off and recrystallized from 50-60% alcohol. The product separated as large, bright yellow needles. The yield was 53.6 g (99% of theoretical). Melting point: 71.5°C . Haberman (37) gives the melting point as $70-71^{\circ}\text{C}$.

2-Nitro-4-methoxyphenol (55)

40 g of 2-nitro-1,4-dimethoxybenzene were placed in a 1500 ml flask and a solution of 80 g of NaOH dissolved in 700 cc of water was added. The mixture was refluxed for 60 hours and 400 cc of water were added. The mixture was steam distilled until no further unreacted diether distilled over. The residue was cooled to room temperature and acidified with concentrated hydrochloric acid. The color changed from a deep red to orange and considerable solid separated. The solid was filtered off and recrystallized from 50% alcohol. The phenol crystallized as large, brilliant orange prisms. The yield was 32 g (87% of theoretical). Melting point: 79.2°C .

Kauffman and Fritz (55) give the melting point as 80° C.

2-Nitro-4-methoxyphenoxyacetic Acid

20 g of 2-nitro-4-methoxyphenol, 12.3 g of chloroacetic acid and 21 g of 50% sodium hydroxide were placed in a 500 ml round-bottom flask and 100 cc of water was added. The flask was arranged for distillation and the mixture slowly distilled until the residue was neutral to litmus (ca. 2 hours; 60-70 cc water distilled over). Half the above quantities of alkali and chloroacetic acid and 50 cc of water were then added and the solution distilled again until neutral. After strongly acidifying with hydrochloric acid the solution was cooled, precipitating the acid. The compound was recrystallized from alcohol. The yield being 13 g (48% of theoretical) of golden-yellow needles. Melting point: 149-150° C. No literature value was found.

Analysis. Calculated for $C_9H_9NO_6$: N, 6.17. Found: 6.21.

Neutralization equivalent. Calculated: 227.2; found: 226.8.

V. DISCUSSION OF EXPERIMENTAL RESULTS

The data for 17 compounds are listed in table II. The value given is the percentage increase (or decrease) relative to the growth increase of the control. With a standard deviation of 0.14 units and an average control growth of 0.49 units, any value less than $\pm 29\%$ could not be considered statistically significant. The value of the standard deviation (0.14 units) is an average of the values for all of the control runs. Calculation of the relative percentage increase or decrease was made using the average value of the controls run during the testing of the compound in question. It is realized that the assigned value of 29% for statistical significance is not a rigid one, but variance from this value would not be great. In a review by Wain (116) it is stated that with the use of the Avena coleoptile straight growth test, any growth deviation in excess of 5% of the control may be considered significant. Testing all of the compounds at once would have been impractical due to the large number of coleoptile sections which would be required. Tables IV and V are typical examples of the measurements recorded during a test run. Table III gives the measurements obtained for the controls run simultaneously with these two compounds.

The activity of the substituted phenylhydrazonoacetic acids is greater than the corresponding propionic acid derivatives. This would indicate, as in the phenoxyalkylcarboxylic acids, that alpha substitution decreases activity. There are, however, results which are not in common with any of the other series of growth regulators: (a) the parent acids show the greatest activity, (b) ethyl 2,4-dichlorophenylhydrazonoacetate is active while the free acid behaves as an inhibitor at all tested concentrations, and (c)

α -(p-bromophenylhydrazono)-propionic acid and α -(p-chlorophenylhydrazono)-propionic acid are highly inhibitory, at all concentrations.

The substituted ethyl acrylate series and the Schiff bases of β -keto acids may show promise with further study. The need for more investigation on the two main series is also apparent.

The data obtained for 2-nitro-4-methoxyphenoxyacetic acid, although not statistically significant, indicates that there may be some combination of substituents and positions which could parallel the effects of the 2,4-dichloro substitution; 2-methoxy-4-nitrophenoxyacetic acid may prove to be more promising.

Since it is possible for the phenylhydrazono-acetic and propionic acids to exist as cis-trans isomers, some of the discrepancies noted may be attributed to the presence of different isomeric states. As has been pointed out, only the cis-isomer of previously investigated cis-trans pairs exhibit activity (the geometrical structure being known in these cases (106,111)). The phenylhydrazonoacetic acid prepared is the higher melting of the two reported isomers (21) and is probably the trans-isomer (in most cases the trans-isomer has the higher melting point). If the phenylhydrazonoacetic acid does have the trans-configuration, this result is in direct contrast to the findings cited above. Little work has been done towards establishing the geometrical structure of these compounds; such information would be of great importance in future investigations of these compounds.

Table II

Percentage Growth Relative to Control

Compound Tested	Concentration x 10 ⁴ molar				
	0.1	0.5	1.0	2.0	5.0
2,4-Dichlorophenoxyacetic acid*		+67	+98	+60	+19
Phenylhydrazonoacetic acid*	+98	+95	+33	-16	
α -phenylhydrazonopropionic acid*	+51	-18	-16	-48	
α -(2-chlorophenylhydrazono)-propionic acid*		-12	-10	-27	
α -(4-chlorophenylhydrazono)-propionic acid*	-89	-90	-68	-82	-82
α -(2,4-dichlorophenylhydrazono)-propionic acid*		-20	-14	-6	
α -(4-bromophenylhydrazono)-propionic acid*		-75	-78	-72	-75
α -(4-methylphenylhydrazono)-propionic acid*		-24	-32	-27	
α -(4-nitrophenylhydrazono)-propionic acid*		-19	-12	-14	
Ethyl 4-chlorophenylhydrazonoacetate#		-7	-10	-44	
Ethyl 2,4-dichlorophenylhydrazonoacetate#		+63	+22	+24	
2,4-Dichlorophenylhydrazonoacetic acid*	-74	-63	-74	-85	-88
Ethyl 2,5-dichlorophenylhydrazonoacetate#		+29	+16	+2	
Ethyl β -(4-chlorophenylamino)-acrylate#		+6	+39	+20	
Ethyl β -(4-methylphenylamino)-acrylate#		14	-3	-8	
α -Phenyliminopropionic acid	0	+31	3	16	
2-Nitro-4-methoxyphenoxyacetic acid	-17	-28	-7	+22	

* The sodium or potassium salt was used.

An emulsion with EMCOL H-86c was used.

Table III

Growth of Avena Coleoptile Sections in Distilled Water

	Relative Length			
Control Number	1	2	3	
	8.28	8.30	8.30	
	8.20	8.30	8.32	
	8.18	8.40	8.45	
	8.40	8.10	8.10	
	8.25	8.15	8.30	
	8.10	8.25	8.32	
	8.25	8.20	8.10	
	8.20	8.45	8.30	
	8.42	8.30	8.35	
	8.15	8.20	8.43	
TOTAL				248.05
Average				8.27
Standard Deviation				0.11

Table IV

Growth of Avena Coleoptile Sections in Solutions
of Sodium Phenylhydrazonoacetate

Concentration x 10 ⁴ molar	2.0	1.0	0.5	0.1
Measured length of coleoptile sections	8.35	8.52	8.40	9.00
	7.70	8.30	9.40	8.56
	8.00	8.10	9.80	8.70
	8.30	8.60	8.20	8.70
	8.30	8.70	9.08	8.90
	8.05	8.75	9.00	8.90
	7.88	8.50	8.13	8.20
	8.10	8.50	9.00	9.30
	8.15	8.30	9.10	8.60
	8.87	8.45	8.40	9.80
Total	81.70	84.72	88.51	88.66
Average	8.17	8.47	8.85	8.87
Deviation from control	-0.10	+0.20	+0.58	+0.60
Relative percentage increase or decrease	-16	+33	+95	+98

Table V

Growth of Avena Coleoptile Sections in Solutions
of α -Phenyliminopropionic Acid

Concentration x 10 ⁴ molar	2.0	1.0	0.5	0.1
Measured length of coleoptile sections	8.30	8.70	8.20	8.23
	8.20	8.50	8.10	8.20
	8.15	8.30	8.60	8.95
	8.55	8.12	8.45	8.25
	7.90	8.00	8.10	7.95
	8.00	7.98	9.60	8.40
	8.10	8.20	8.60	8.25
	7.90	7.95	8.10	8.05
	8.40	8.46	8.89	8.20
	- - *	8.28	7.92	8.20
Total	73.50	82.49	84.60	82.68
Average	8.17	8.25	8.46	8.27
Deviation from control	-0.10	-0.02	+0.19	0.00
Relative percentage increase or decrease	-16	-3	+31	0

* Coleoptile section dead.

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