

CONFORMATIONAL ANALOGS OF SOME PHYTOACTIVE COMPOUNDS

THESIS

Presented to the Graduate Council of the  
North Texas State University in Partial  
Fulfillment of the Requirements

For the Degree of

MASTER OF SCIENCE

By

Wm. Paul Skelton, B.A.

Denton, Texas

August, 1973

115

Skelton, Wm. Paul, Conformational Analogs of Some Phytoactive Compounds. Master of Science (Chemistry), August, 1973, 27 pp., references, 27 titles.

The plant growth regulating activity of a recently studied series of monoanilides of aliphatic dicarboxylic acids indicated that the most active analog was *p*-chloroglutaranilic acid. In an effort to determine if there is a specific conformational structure which is most effective at the appropriate active physiological site, the synthesis of a group of sterically restricted analogs was undertaken. A portion of the polymethylene carbon skeleton of glutaric acid was replaced by selected aromatic carbons in benzenedicarboxylic acids to produce a series of ridged conformers, and the relative plant growth regulating properties of these derivatives were determined.

Several phthalic acid compounds have previously been reported to possess phytoactivity; however, the analogous iso- and terephthalanilic acid derivatives have either not been synthesized or have not been reported as being tested in plant growth systems. Accordingly, the several monoamide isomers containing a phenyl-, *p*-chlorophenyl-, or 2,4-dichlorophenyl structure were prepared and studied for plant growth regulating activity.

Phthalanilic and 4'-chlorophthalanilic acids were synthesized by hydrolysis of the appropriate imide which was

formed through a thermal condensation between phthalic anhydride and the appropriate amine. 2',4'-Dichlorophthalanilic acid was obtained directly by aminolysis of phthalic anhydride with 2,4-dichloroaniline. Isophthalanilic and 2',4'-dichloroisophthalanilic acids were prepared by direct action of m-(chloroformyl)benzoic acid and the corresponding amine. 4'-Chloroisophthalanilic acid was synthesized by the condensation of methyl m-chloroformylbenzoate with p-chloroaniline, followed by hydrolysis of the ester to produce the free acid. The terephthalanilic acid analogs were synthesized by reacting terephthalic anhydride with the appropriate amine. The structure of these derivatives were confirmed by elemental analysis, infrared, and proton magnetic resonance studies.

Bioassays on these structural analogs were carried out using hydroponic assay techniques. Sunflower seeds were permitted to germinate and grow in the presence of water for seven days, and the aqueous media was then replaced by the various test solutions. The biological effect observed was based on the differential growth between the height of the plant at seven days and seventeen days, as compared with sunflower seedlings grown in water alone.

Based on the data presently available, it appears that the haloanilides of the benzene dicarboxylic acids are approximately one-tenth as active in promoting shoot growth of sunflower as the p-chloroglutaranilic acid derivative. For example, the latter compound substantially increased the

rate of growth at a concentration level of 1 mg/liter, and the corresponding p-chloroanilides of the ortho, meta, and para-isomers produced comparable effects, respectively, at 10 mg/liter. Since all of the derivatives possess phytoactivity, and since these effects are augmented by the presence of halogen on the aromatic ring system, it may be that the phytoactivity is not produced by any specific ridged conformation.

CONFORMATIONAL ANALOGS OF SOME PHYTOACTIVE COMPOUNDS

THESIS

Presented to the Graduate Council of the  
North Texas State University in Partial  
Fulfillment of the Requirements

For the Degree of

MASTER OF SCIENCE

By

Wm. Paul Skelton, B.A.

Denton, Texas

August, 1973

## TABLE OF CONTENTS

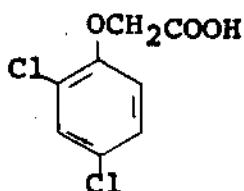
	Page
LIST OF TABLES . . . . .	iv
INTRODUCTION . . . . .	1
EXPERIMENTAL . . . . .	5
Organic Synthesis . . . . .	5
Phthalanilic Acid . . . . .	5
4'-Chlorophthalanilic Acid . . . . .	6
2',4'-Dichlorophthalanilic Acid . . . . .	6
Isophthalanilic Acid . . . . .	6
4'-Chloroisophthalanilic Acid . . . . .	6
2',4'-Dichloroisophthalanilic Acid . . . . .	7
Terephthalanilic Acid . . . . .	8
4'-Chloroterephthalanilic Acid . . . . .	8
2',4'-Dichloroterephthalanilic Acid . . . . .	8
Biological Assay Procedures . . . . .	9
Cultivated Plants . . . . .	9
Preparation of Test Compounds . . . . .	9
RESULTS AND DISCUSSION . . . . .	10
BIBLIOGRAPHY . . . . .	25

## LIST OF TABLES

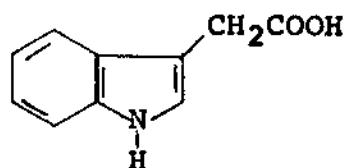
Table	Page
I. Infrared Spectral Data of Some Phthalanilic Acids . . . . .	14
II. Nuclear Magnetic Resonance Spectral Data of Some Anilides of Benzenedicarboxylic Acid . .	15
III. Infrared Spectral Data of Some Isophthalanilic Acids . . . . .	18
IV. Infrared Spectral Data of Some Terephthalanilic Acids . . . . .	21
V. Effect on Shoot Elongation of Sunflower Seedling Treated with Isomers of Phthalanilic Acid . . . . .	23

## INTRODUCTION

For many years extensive efforts have been made to control plant growth. Many natural and synthetic derivatives have been examined, and among the more widely studied compounds may be included 2,4-dichlorophenoxyacetic acid (2,4-D) (27) and indole-3-acetic acid (IAA) (8).



(2,4-D)



(IAA)

The structure-activity relationships of such compounds as plant growth regulatory agents has been extensively reviewed (23,21). For example, the bioactivity of many indole- and aryl-alkane carboxylic acids have been examined, and Koepfli has itemized structural criteria which he proposes must be possessed by bioactive compound for maximum activity (7). Briefly, these are a ring with at least one double bond and a carboxyl group on the side chain with at least one carbon atom between the ring and the carboxyl group.

Structural variations in the unsaturated ring portion of the aryoxalykane carboxylic acid have been examined, and

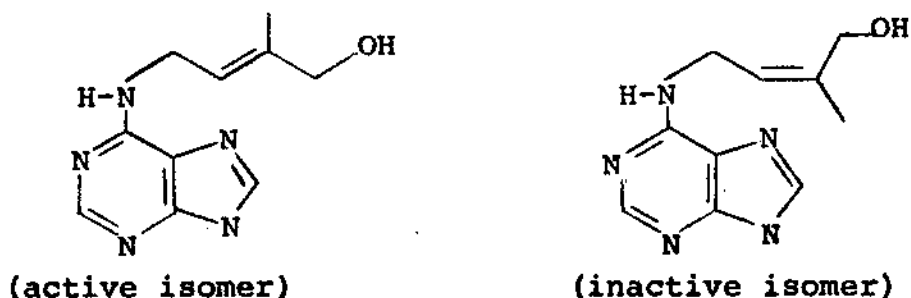


it was found that the presence and position of a substituent group was important. For example, enhanced biological activity was observed when chlorine was substituted at the 2-, 4-, and 2,4- positions. Corresponding analogs with chlorines in the 3,5- or 2,6- positions were shown to be inactive, while the 2,3-, 2,4-, 2,5-, and 3,4- positions showed activity (10,24).

Benzoic acid (7) and its halo derivatives have been examined for herbicidal properties, and in the Avena straight coleoptile test, the 2-, 2,5-, and 2,6- chlorinated benzoic acids proved to be the more active, whereas the 2,4- disubstituted derivative was inactive (13). Further, trisubstituted chlorobenzoic acids proved to be biologically active in the pea test (22,1) and Avena test (1). Attempts have been made to explain the activity of 2,6- and 2,3,6- chlorobenzoic acids on the basis of steric factors (22). For example, the carboxyl group is pushed out of the plane of the ring by the chlorines in the 2,6- compound, which may explain why 2,3,6- homolog is active. However, this explanation does not explain the biological inactivity of the 2,4,6- compound, since, based on the latter assumption, it should be active. In passing, 2,3,6-trichlorobenzoic acid is used widely today as a herbicide (2).

That steric effects do play an important role in the manner that compounds affect plant growth is evidenced by the fact that in aryloxyalkanecarboxylic acids which possess an

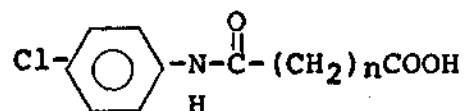
asymmetric carbon atom, only one of the isomers demonstrates plant growth activity (20). More recently several geometric isomers of zeatin have been prepared and specific analogs have been shown to increase plant growth, whereas the corresponding isomer did not (18).



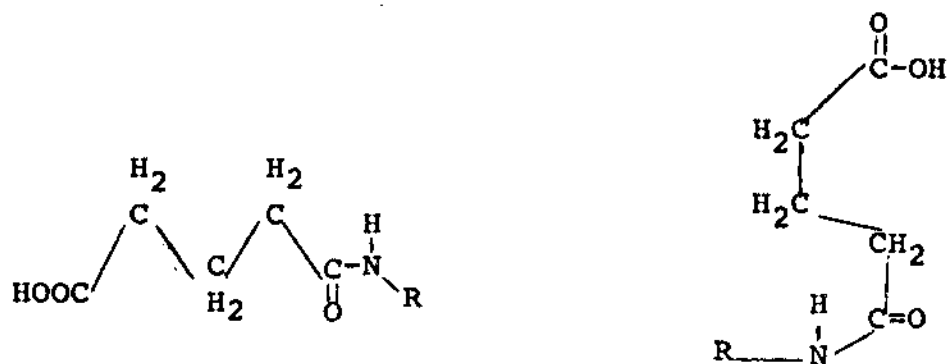
Based on chemical structure, there are two general types of herbicidal anilines. In one type, the chloroacetamide nitrogen is attached to a phenyl and/or alkyl group; in the other the aniline nitrogen has only hydrogens attached. Examples of some commercially important aniline derivatives in use today include: 2-chloro-2,6-diethyl-N-(methoxymethyl)-acetanilide, 2-chloro-N(isopropyl)-acetanilide, 2-chloro-N-(1-methyl-2-propynyl)acetanilides, 3,4-dichloropropionanilide, and 3,4-dichloro-2-methyl-arylanilide (3).

Recently 4'-chloroglutaranilic acid (CGA) was synthesized and studied for its effect on sunflower as a herbicidal agent (17) and was found to inhibit growth at a concentration of  $10^{-4}$  M. This suppressed rate of growth was greater than that observed for 2,4-D at the same concen-

tration. Homologs of the general structure,



were subsequently prepared, where  $n$  was varied from 3 to 0, and their herbicidal properties were studied at  $10^{-4}$  M concentration in the sunflower system. A decrease in herbicidal activity was observed which coincided with a decrease in the length of the carbon side chain (9), the most active analog being  $n=3$ , *p*-chloroglutaranilic acid. Upon examination of the structure of CGA it is apparent that the aliphatic portion of the molecule can assume several conformations due to free rotation around the  $sp^3$  carbon bond. The synthesis of a series of restricted conformers



might indicate which of these is the most active conformational form of CGA, and suggest the stereochemical configuration of the site of biological activity in vivo. Accordingly, a selected group of monanilides of *o*, *m*, and *p*-benzenedicarboxylic acids were prepared for subsequent study in plant growth assay systems.

## CHAPTER

### ORGANIC SYNTHESSES

All melting points were determined with a Thomas-Hoover capillary melting point apparatus and are uncorrected. Deuterated dimethylsulfoxide was used as a solvent for nuclear magnetic resonance (nmr) spectra, which were determined on a JOEL High Resolution NMR JNM-PS-100. Infrared (ir) spectra were determined using KBr wafer technique on a Perkin-Elmer 237 grating spectrophotometer. The C,H,N analyses were carried out by Midwest Microlab, Indianapolis, Indiana.

Phthalanilic acid. In 100 ml of ethanol was dissolved 5.0 g of N-(phenyl)phthalimide. While stirring, 23 ml of 0.988 N aqueous sodium hydroxide was added, and the resulting mixture was refluxed for two hours (14). The solution was cooled, and added to a large volume of distilled water. The reaction mixture was placed in an ice bath and acidified to pH 1 with concentrated hydrochloric acid to yield a solid product which was filtered and washed with water. The material was recrystallized from ethanol-water solution to yield 1.2 g (22%) of product, mp 178°. The reported mp is 169° (19).

Anal. Calculated for  $C_{14}H_{11}NO_3$ ; C,69.70; H,4.59; N,5.81. Found: C,68.33; H,4.49; N,5.82.

4'-Chlorophthalanilic acid. The same synthetic procedure utilized for the phthalanilic acid derivative was repeated using 5.0 g of N-(p-chlorophenyl)phthalimide and 20.02 ml of 0.988 N sodium hydroxide. The amount of product recovered was 3.6 g (67.4%), mp 203-205°. The reported mp is 187.5° (19).

Anal. Calculated for  $C_{14}H_{10}ClNO_3$ : C, 60.99; H, 3.66; N, 5.08. Found: C, 60.89; H, 3.54; N, 5.09.

2',4'-Dichlorophthalanilic acid. To 200 ml of chloroform was added 9.46 g of phthalic anhydride and 10.37 g of 2,4-dichloroaniline. After stirring for two hours at room temperature, the product which precipitated was filtered and washed with chloroform. There was recovered 5.4 g (28%) of product, mp 155-160°.

Anal. Calculated for  $C_{14}H_9Cl_2NO_3$ : C, 54.22; H, 2.93; N, 4.52. Found: C, 54.07; H, 3.02; N, 4.50.

Isophthalanilic acid. Two grams of m-(chloroformyl) benzoic acid (11) was dissolved in 75 ml of anhydrous ether, and to this was added a solution of 0.99 ml of aniline dissolved in 50 ml of anhydrous ether. The product which formed was filtered and washed with ether to yield 2.1 g (81%) of material with a mp of 285°.

Anal. Calculated for  $C_{14}H_{11}NO_3$ : C, 69.70; H, 4.59; N, 5.81. Found: C, 70.05; H, 4.22; N, 5.95.

4'-Chloroisophthalanilic acid. Using a previously reported procedure (25) 3.31 g of methyl m-chloroformyl-

benzoate and 2.32 ml of triethylamine were dissolved in benzene and to this solution 2.13 g of *p*-chloroaniline was added. The reaction product was filtered, washed with water, and recrystallized from methanol to yield 2.77 g (58%), mp 152°. The resulting amide was dissolved in 65 ml of methanol containing 0.38 g of sodium hydroxide, and the mixture was heated under reflux for five hours. The cooled solution was added to 400 ml of water, filtered, and the filtrate was placed in an ice bath and acidified to pH 1 with concentrated hydrochloric acid. The product which precipitated was washed with water and dried to yield 1.13 g (43%) of material, mp 295°.

Anal. Calculated for  $C_{14}H_{10}ClNO_3$ : C, 60.99; H, 3.66; N, 5.08. Found: C, 61.01; H, 3.81; N, 5.08.

2',4'-Dichloroisophthalanilic acid. The procedure utilized for this synthesis was the same as that for isophthalanilic acid using 2.62 g of *m*-(chloroformyl)benzoic acid and 2.3 g of 2,4-dichloroaniline. The amount of product recovered was 2.18 g (50%), mp 227°.

Anal. Calculated for  $C_{14}H_9Cl_2NO_3$ : C, 54.22; H, 2.93; N, 4.52. Found: C, 53.92; H, 3.03; N, 4.41.

Terephthalic anhydride. A solution of 15 g of terephthaloyl chloride (11) and 1.33 ml of water in 300 ml of anhydrous ether was refluxed for 45 hours. The resulting mixture was evaporated to dryness, and the solid was washed

with benzene and finally with petroleum ether. There was recovered 10.9 g (80%) of the product which decomposed at 200° (11).

Terephthalanilic acid. A mixture of 3 g of terephthalic anhydride and 1.47 ml of aniline was stirred in ether. The resulting product was filtered and recrystallized from hot nitrobenzene. After filtering the product was refluxed in ether for four hours to remove traces of nitrobenzene. Recovered was 1.81 g (83.4%), mp 315°.

Anal. Calculated for  $C_{14}H_{11}NO_3$ : C, 69.70; H, 4.59; N, 5.81. Found: C, 69.98; H, 4.61; N, 5.69.

4'-Chloroterephthalanilic acid. Using the same general procedure as previously described for the synthesis of terephthalanilic acid, 3 g of the terephthalic anhydride and 2.067 g of p-chloroaniline were condensed to yield 2.07 g of product (84%), mp 315°.

Anal. Calculated for  $C_{14}H_{10}ClNO_3$ : C, 60.99; H, 3.66; N, 5.08. Found: C, 60.78; H, 3.55; N, 5.02.

2',4'-Dichloroterephthalanilic acid. A mixture of 2.64 g of 2,4-dichloroaniline and 3 g of terephthalic anhydride was condensed as previously described, and the product was recrystallized from ethanol to yield 2.07 g of material (74.1%), mp 265-267°.

Anal. Calculated for  $C_{14}H_9Cl_2NO_3$ : C, 54.22; H, 2.93; N, 4.52. Found: C, 54.54; H, 3.01; N, 4.58.

## BIOASSAY METHODS

Hydroponic Studies. All growth studies were carried out in diSPo No. B1220 Seed-Pak growth pouch and the following procedure was followed: Untreated sunflower (Helianthus annus, L.) seeds were planted and 20 ml of deionized water was added. Following the addition of water the plants were placed as close as possible to fluorescent lamps. After three days the pouches were examined for the presence of ungerminated seeds which were removed; also, seed husks were removed from germinated seeds. At this time the plants were moved a distance of 5 cm from the fluorescent lamps and rotated 180° every day for homogeneous light contact. On the seventh day after planting the pouches were drained, and to these were added 30 ml of test solution. Five days after treatment with test solutions, 20 ml of nutrient solution was added to the pouches (12). Control plants were given only water and nutrients. Ten days after treatment of the plants with test solution the plants were measured from the top edge of the seed trough to the stem meristem apex. Plant heights were averaged and related to control heights.

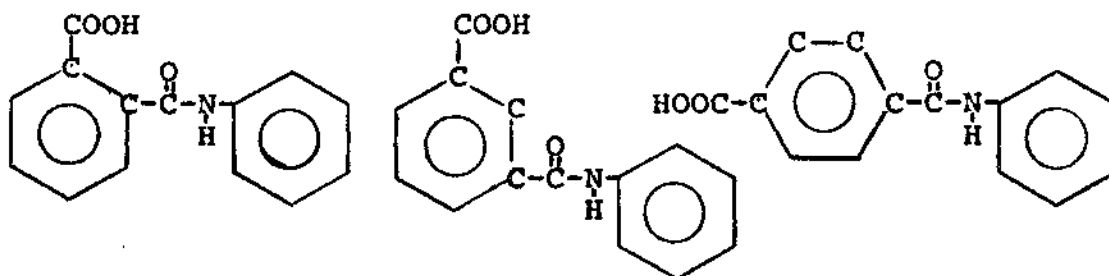
Preparation of Test Compound Solutions. Each compound was tested at concentrations of 10 mg per l and 1 mg per l. The solutions were prepared as follows: 0.01 g of test



compound was dissolved in a liter of deionized water with the aid of two drops of Tween 20 (polyoxyethylene(20)sorbitan monolaurate). This was a stock solution which was diluted to the desired concentration. Test solutions were applied with pipets (9).

### RESULTS AND DISCUSSION

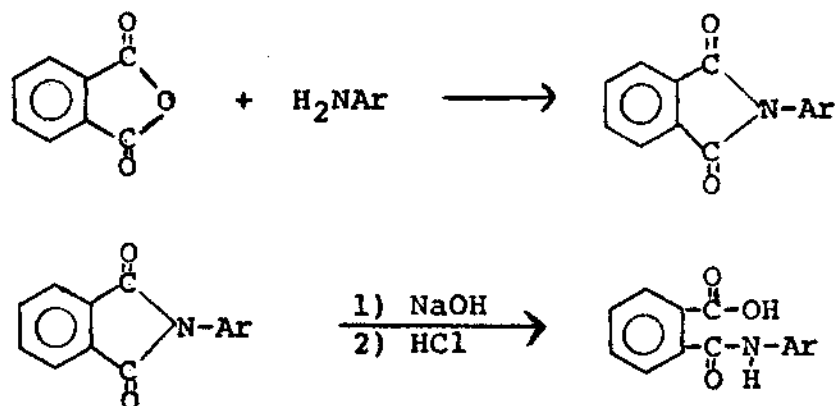
The plant growth regulating activity of a series of monoanilides of aliphatic dicarboxylic acids indicated that the most active analog was *p*-chloroglutaranilic acid (9). In an effort to determine if there is a specific conformational structure which is most effective at the appropriate active physiological site, the synthesis of a group of sterically restricted conformers would be of interest for study in comparative biological systems. For example, a portion of the polymethylene carbon skeleton of glutaric acid may be replaced by each of the three benzene-dicarboxylic acids to produce a series of ridged conformers whose relative plant growth regulating properties might define the structural requirements to produce plant growth activity in anilides of dicarboxylic acids.



Of further interest is the fact that several phthalic acid compounds have previously been reported to possess phyto-activity. For example, using tomato plants as the assay system 2'-, 3'-, and 4'-chlorophthalanilic acids were found to stimulate fruit production at lower concentration levels, while at 2000/ppm injury to the plants was observed (6). Ryshka has reported that 3'-chlorophthalanilic acid increases growth in wheat roots (16), and at concentrations of  $10^{-3}$  M, phthalanilic and 4'-chlorophthalanilic acids were reported to give negative geotropism in lentil seedling roots (15). 2',4'-Dichlorophthalanilic acid is reported to have moderate effects on geotropism in Lens esculenta seeds (14).

The analogous iso- and terephthalanilic acid derivatives have either not been synthesized or have not been reported as being tested in plant growth systems. Accordingly, the several monoamide isomers containing a phenyl-, p-chlorophenyl-, or 2,4-dichlorophenyl-structure were prepared and studied for plant growth regulating activity.

In an attempt to prepare phthalanilic and 4'-chlorophthalanilic acids directly through the aminolysis of phthalic anhydride by the appropriate amine, only the corresponding imides could be isolated. However, the desired analog could readily be obtained from the imide via controlled alkaline hydrolysis followed by acidification to precipitate the product.

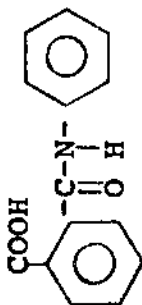


The resulting carboxyanilides of phthalic acid were easily distinguished from the imides by melting point comparisons, and in several instances the observed melting points were higher than those reported in the literature for the mono acids. Phthalanilic acid prepared in these studies melted at  $178^\circ$ , while its reported value is  $169^\circ$  (19), and *p*-chlorophthalanilic acid melted at  $203-205^\circ$ , as compared to the reported value of  $187.5^\circ$  (19). Spontaneous cyclization of these phthalanilic acids to the imides by heat produced the anticipated imides. This was accomplished by heating the free acid anilides quickly in a melting point apparatus to its melting point and then removing the sample until it solidified. A second melting point determination produced a change in the melting point which corresponded to that of the imide. Elemental analysis for C, H, and N also confirmed the empirical formula of the imides and acids. The spectra of the imides contained a unique peak at  $5.6 \mu$  which was not

present in the spectra of the free acids, and the ir spectra of phthalanilic and p-chlorophthalanilic acids were quite similar (Table I). The nmr spectra also served to distinguish the free acids from the imides. A method previously described by Zanger (26) was used to help assign the chemical shifts for the different anilides. These data are summarized in Table II, and the observed and calculated values are in good agreement. Integration of the individual spectras revealed 11 hydrogens for phthalanilic acid and 10 hydrogens for p-chlorophthalanilic acid.

In contrast to the isomers described above, 2',4'-dichlorophthalanilic acid was produced directly from the interaction of phthalic anhydride and 2,4-dichloroaniline. The melting point of the acid was so similar to that reported for the imide that the melting point technique previously described to distinguish between such structures was unsatisfactory; i.e., the melting point reported for the imide is 154-155° (14), and the acid 155-160°. The imide was synthesized and its ir and nmr spectras were compared to those of the anilide. An absorption maxima was not observed at 5.6  $\mu$  in the ir spectrum of the anilide, whereas a spectrum of the imide did possess a  $\lambda_{\max}$  in this region. Table I summarizes the values observed for the absorption maxima of the free acid and the imide. The nmr spectrum of the anilide integrated

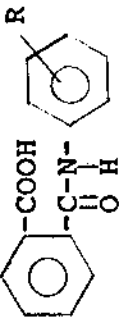
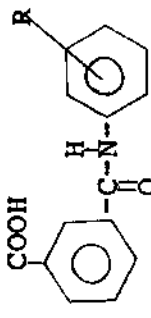
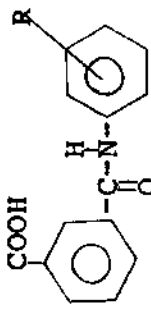
TABLE I  
 INFRARED SPECTRAL DATA OF SOME PHTHALANILIC ACIDS\*



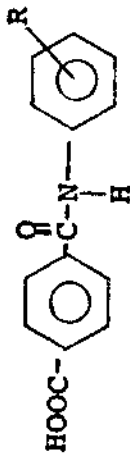
Compound	Wavelength (Microns)
Phthalanilic Acid	3.05(sh), 3.30(b), 5.85(s), 6.10(b), 6.50(s), 6.95(s)
4'-Chlorophthalanilic Acid	3.05(sh), 3.28(b), 5.85(s), 6.10(b), 6.29(sh), 6.50(s)
2',4'-Dichlorophthalanilic Acid	3.03(sh), 3.30(b), 5.83(s), 6.15(b), 6.30(sh), 6.55(s)
N-(2,4-dichlorophenyl)-phthalimide	5.61(sh), 5.72(sh), 5.85(s), 6.75(s), 7.20(s)

\*All spectra were determined on a Perkin-Elmer 237 grating spectrophotometer using KBr wafers.

TABLE II  
 NUCLEAR MAGNETIC RESONANCE SPECTRAL DATA OF SOME ANILIDES  
 OF BENZENEDICARBOXYLIC ACIDS\*

Compound	Absorption ( $\delta$ )
	6.99 (d,1), 7.20 (s,2), 7.40 (d,2), 7.50 (t,2), 7.80 (dd,2), 10.2 (s,1)
	7.30 (d,2), 7.40 (dd,4), 7.70 (d,2), 10.2 (s,1)
	7.40 (dd,1), 7.50 (s,1), 7.60 (m,2), 7.70 (dd,2), 7.90 (d,1), 10.0 (s,1)

Isophthalanilic Acid	7.02 (t,1), 7.30 (t,2), 7.60 (t,1), 7.80 (d,2), 8.18 (t,2), 8.50 (s,1)
4'-Chloroisophthalanilic Acid	7.50 (dd,4), 7.66 (t,1), 8.10 (d,2), 8.50 (s,1), 10.50 (s,1)
2',4'-Dichloroisophthalanilic Acid	7.30 (dd,1), 7.50 (s,1), 7.59 (d,1), 7.60 (s,1), 8.10 (d,2), 8.50 (s,1), 10.5 (s,1)



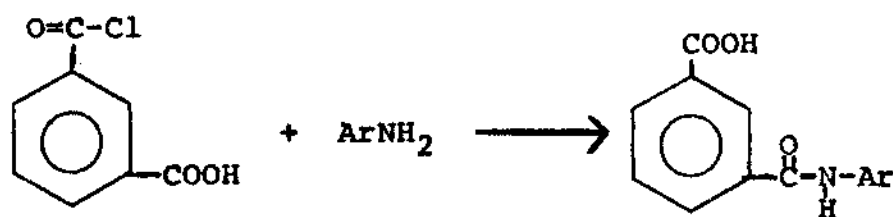
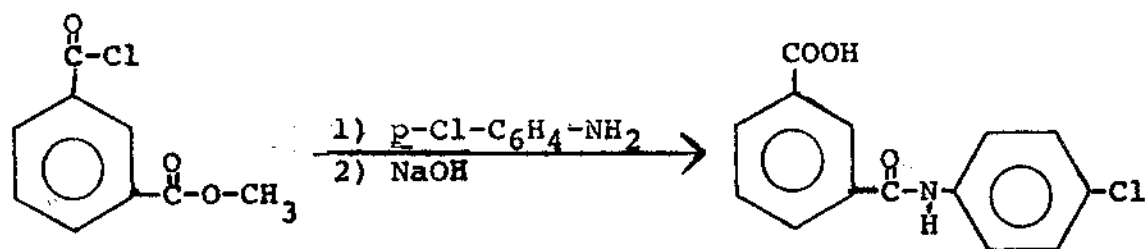
Terephthalanilic Acid	7.10 (t,1), 7.29 (t,2), 7.80 (d,2), 8.20 (s,4), 10.49 (s,1)
4'-Chloroterephthalanilic Acid	7.55 (dd,4), 8.00 (s,4), 10.4 (s,1)
2',4'-Dichloroterephthalanilic Acid	7.30 (dd,1), 7.42 (s,1), 7.58 (s,1), 8.00 (s,4), 10.10 (s,1)

---

\*NMR spectra were determined on a JOEL High Resolution NMR JNM-PS-100 using deuterated dimethylsulfoxide as the solvent and internal standard.

for 9 hydrogens, while that of the imide for 7 hydrogens, and these data are presented in Table II.

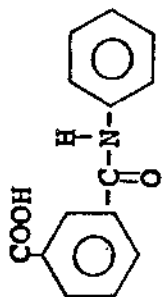
*p*-Chloroisophthalanilic acid was obtained by reacting methyl *m*-chloroformylbenzoate with *p*-chloroaniline followed by alkaline hydrolysis of the ester. Isophthalanilic and 2',4'-dichloroisophthalanilic acids were prepared directly from *m*-chloroformylbenzoic acid and the appropriate amine.



The empirical formulas of the anticipated anilides was confirmed by elemental analysis. The ir spectra (Table III) of the three anilides were very similar with each having absorption maxima as indicated: NH (6.53 - 6.6  $\mu$ ), C=O (5.95), and OH (3.8 - 3.9). Using nmr techniques, chemical shifts were calculated (26) and compared to those observed



TABLE III  
 INFRARED SPECTRAL DATA OF SOME ISOPHTHALANILIC ACIDS\*

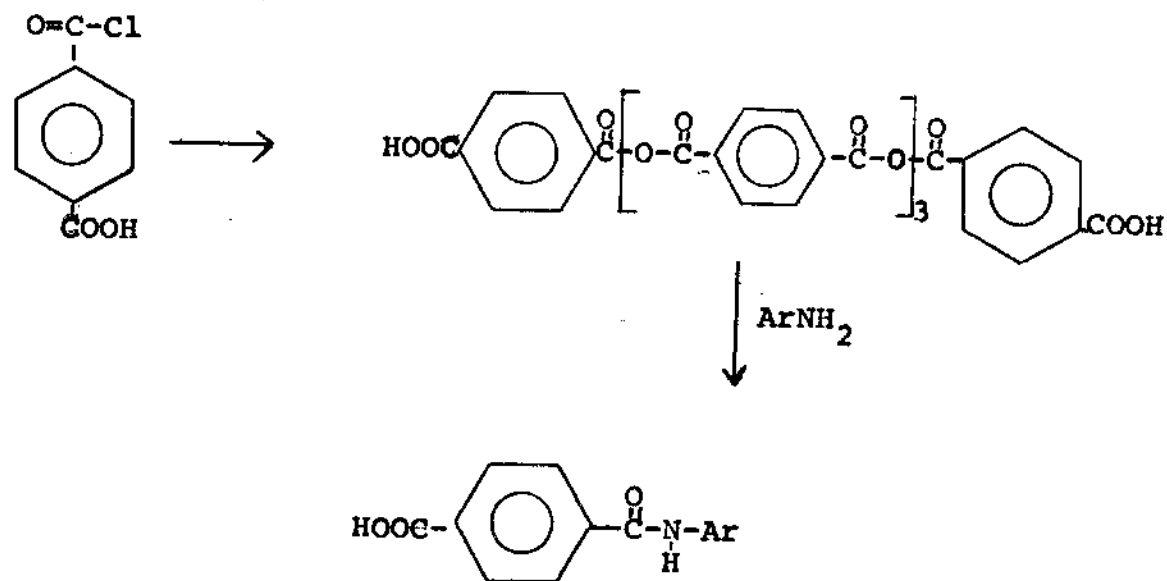


Compound	Wavelength (Microns)
Isophthalanilic Acid	3.06(sh), 3.50(b), 3.90(b), 5.95(s), 6.10(sh), 6.29(sh), 6.53(sh), 6.95(sh)
4'-Chloroisophthalanilic Acid	3.08(sh), 3.30(b), 3.80(b), 5.95(s), 6.10(sh), 6.29(sh), 6.55(sh), 7.15(sh)
2',4'-Dichloroisophthalanilic Acid	3.10(sh), 3.35(b), 3.90(b), 5.94(s), 6.08(sh), 6.33(s), 6.60(s), 6.95(sh)

\*All spectra were determined on a Perkin-Elmer 237 grating spectrophotometer using KBr wafers.

in the actual spectra, and the data exhibited good agreement. For isophthalanilic acid there was calculated 11 hydrogens, 10 hydrogens for *p*-chloroisophthalanilic acid, and 9 hydrogens for 2',4'-dichloroisophthalanilic acid from nmr spectral data (Table II).

An attempt to prepare the terephthalanilic acid analogs directly from *p*-chloroformylbenzoic acid failed. It was found that upon attempted synthesis, the acid chloride spontaneously polymerizes to form the corresponding anhydride (5). However, the desired analogs was ultimately obtained by interaction of the anhydride with the appropriate amine.



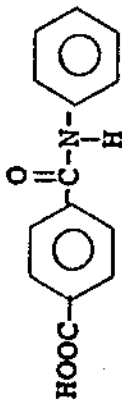
Elemental analysis confirmed the empirical formula predicted for the monoanilide derivative. Further, there was observed 11 hydrogens for terephthalanilic acid, 10 hydrogens for *p*-

chloroterephthalanilic acid, and 9 hydrogens for 2',4'-dichloroterephthalanilic acid from the corresponding nmr spectra (Table II). The ir spectra of the anilides also confirmed the correct free acid (Table IV).

Bioassays on these various structural analogs of p-chloroglutaranilic acids were carried out using hydroponic assay techniques under conditions which were comparable to the previously reported studies (9). Sunflower seeds were permitted to germinate and grow in the presence of water for seven days, and the aqueous media was then replaced by the various test solutions. The data recorded in Table V is based on the differential growth between the height of the plant at seven days and seventeen days, as compared with sunflower seedlings grown in water alone. At the completion of the experiment the values for the plant heights were submitted to a computer program in an effort to evaluate the standard deviation (4). However, the data were so erratic that the numerical figures did not appear to be reliable. Accordingly, these data are not reported in this study; rather, only qualitative effects are presented. Further studies are presently in progress in an effort to qualitate these biological responses in the sunflower system.

Anticipating the inherent problems associated with measuring the effect of chemical treatment on plant growth, four replicate studies were carried out over a period of approximately four months. The growth data obtained proved

TABLE IV  
 INFRARED SPECTRAL DATA OF SOME TEREPHTHALANILIC ACIDS\*



Compound	Wavelength (Microns)
Terephthalanilic Acid	3.02 (sh), 3.40 (b), 3.90 (b), 5.97 (sh), 6.05 (sh) 6.29 (sh), 6.59 (s), 6.97 (s)
4'-Chloroterephthalanilic Acid	3.10 (sh), 3.50 (b), 3.90 (b), 5.95 (s), 6.10 (sh), 6.30 (s), 6.75 (s)
2',4'-Dichloroterephthalanilic Acid	3.09 (sh), 3.35 (b), 3.90 (b), 5.90 (s), 6.10 (sh), 6.35 (sh), 6.60 (s), 7.05 (sh)

\*All spectra were determined on a Perkin-Elmer 237 grating spectrophotometer using KBr wafers.

to be quite erratic, and a composite summary of only three of the tests are included in Table V. A part of this difficulty was due to the inability to control light and temperature effects; however, these problems have recently been overcome, and additional studies are currently in progress in these laboratories in an effort to critically evaluate the biological effects of these derivatives on sunflower growth. Based on the data presently available, it appears that the haloanilides of the benzene dicarboxylic acids are approximately one-tenth as active in promoting shoot growth of sunflower as the *p*-chloroglutaranilic acid derivative. For example, the latter compound substantially increased the rate of growth at a concentration level of 1 mg/l, and the corresponding *p*-chloroanilides of the ortho, meta, and para- isomers produced comparable effects, respectively, at 10 mg/liter.

At the 10 mg/liter concentration it is interesting to speculate on the results of these preliminary biological assays with the non-halogenated derivatives of the benzene-carboxylic anilides. Of the three isomers, isophthalic acid was three times as active in stimulating growth as were the *ortho* and *para* isomers. This suggests that the more physiologically active conformation of the aliphatic dicarboxylic anilides might be one in which the carboxyl and anilide moieties are in an intermediate structural conformation on the active sites rather than being either tightly coiled (ortho structure) or in a linear

TABLE V  
EFFECT ON SHOOT ELONGATION OF SUNFLOWER SEEDLING  
TREATED WITH ISOMERS OF PHTHALANILIC ACID<sup>a</sup>

Compound <sup>b</sup>	Growth in height relative to control <sup>c</sup>	
	<u>1 mg/l</u>	<u>10 mg/l</u>
Phthalanilic Acid	0	++
4'-Chlorophthalanilic Acid	++	++++
2',4'-Dichlorophthalanilic Acid	+++	+++
Isophthalanilic Acid	++	+++
4'-Chloroisophthalanilic Acid	+++	++++
2',4'-Dichloroisophthalanilic Acid	+++	++++
Terephthalanilic Acid	++	+
4'-Chloroterephthalanilic Acid	0	++
2',4'-Dichloroterephthalanilic Acid	+++	++++
4'-Chloroglutaranilic Acid	++++	0

<sup>a</sup>Data for each test is an average of values from three replications consisting of 15 plants per replication.

<sup>b</sup>Test compounds were added to roots of 7 day old sunflower seedlings grown in hydroponic media. Measurements were made ten days after application of the test compounds, and data represents change in growth between 7 and 17 day.

<sup>c</sup>Average height of control plants at termination of experiment, 1.3 cm.

form (para isomer). Since all of the derivatives produced some effect, and since these effects were augmented by the presence of halogen on the aromatic ring system, it may be that the phytoactivity is not produced by any specific ridged conformation.

In general, the introduction of a halogen in the anilide portion of the molecule increases the phytoactivity of the analog. This is in agreement with the previous observations concerning the biological activity of aryloxy-alphatic acid and their halo derivatives (24).

Further studies on these derivatives both in hydroponic media and using avena coleoptile assay techniques may more clearly indicate the biochemical function of these plant growth factors.

## REFERENCES

1. Bentley, J. A., "Growth Regulating Effects of Certain Organic Compounds," Nature 165, 449 (1950).
2. Brady, N. C., Advances in Agronomy, New York and London, Academic Press, Inc., 1972, pp. 328-331.
3. Brady, N. C., Advances in Agronomy, New York and London, Academic Press, Inc., 1972, pp. 368-371.
4. Fritz, James and Schenk, George, Quantitative Analytical Chemistry, Boston, Allyn and Bacon, Inc., 1966, pp. 328-329.
5. Gevaert Photo-Producten N.V., Belg. 545, 313 (1956), Chem. Abstr. 54, 20316c (1960).
6. Hoffmann, O. L., and Smith, A. E., "A new group of plant-growth regulators," Science 109, 588 (1949).
7. Koepfli, J. B., Thimann, K. V., and Went, F. W., "Phytohormones. Structure and physiological activity I.," J. Biol. Chem. 122, 763-780 (1938).
8. Kögl, F., Haagen-Smit, A. J., and Erxleben, H., "Über ein neuex Auxin (Hetero-auxin) aus Harn. XI.," Physiol. Chem. 228, 90 (1934).
9. Larsen, S. P., "Effects of 4'-chloroglutaranilic acid on growth and development of sunflower seedlings," Ph.D. dissertation, Department of Chemistry, North Texas State University, Denton, Texas, 1972.
10. Leaper, J. M. F., and Bishop, J. R., "Relation of halogen position to physiological properties in the mono-, di-, and tri-chlorophenoxyacetic acids," Botan. Gaz. 112, 250-258 (1951).
11. Marvel, C. S., Kraiman, E. A., "Polythiolesters. III. Further studies on preparation in emulsions", J. Org. Chem. 18, 1664-1669 (1953).
12. Mitchell, J. W., and Livingston, G. A., Methods of studying plant hormones and growth-regulating substances, Agriculture Handbook No. 336, Supt. of Documents, U. S. Govt. Printing Office, 1968, p. 129.



13. Muir, R. M., and Hansch, C., "The relationship of structure and plant growth activity of substituted benzoic and phenoxyacetic acids," Plant Physiol. 26, 369-374 (1951).
14. Pagani, G., Baruffini, A., Borgna, P., Caccialanza, G., "Various N-substituted phthalamic acids. Effects of root geotropism in germinating seeds of Lens esculenta," Farmaco Ed. Sci. 23, 448-465 (1968).
15. Resplandy, A., "Two cases of structural analogy in substances disturbing plant geotropism," C. R. Acad. Sci., Paris, Ser. D226(12), 1253-1255 (1968); Chem. Abstr. 68, I04030e (1968).
16. Ryshka, F., "Synthesis of N-(30chlorophenyl)phthalamic acid," Sornik Stud. Nauch-Issledovatel. Rabot Moskov. Sel'skokhoz. Akad. im. K.A. Timiryazeva 6, 88-92 (1956); Referat. Zhur., Kim., Abstract 61499; Chem. Abstr. 53, 1234d (1959).
17. Sargent, D. R., "Biological Inhibitors," Ph.D. dissertation, Department of Chemistry, North Texas State University, Denton, Texas, 1971.
18. Schmitz, R. Y., Skoog, F., Playtis, A. J., and Leonard, N. J., "Cytokinins: Synthesis and biological activity of geometric and position isomers of zeatin," Plant Physiol. 50, 702-705 (1972).
19. Sherrill, M. L., Schaffer, F. L., Shoyer, E. P., "The isomerism of phenylphthalimide and a study of the nitro and chloro derivatives," J. Amer. Chem. Soc. 50, 474-485 (1928).
20. Smith, M. S., and Wain, R. L., "The plant growth-regulating activity of dextro- and laevo- $\alpha$ -(2-naphthoxy)propionic acid," Proc. Roy Soc. B139, 118 (1951).
21. Steward, F. C., and Krikorian, A. D., Plants, Chemicals, and Growth, New York and London, Academic Press, Inc. 1971, pp. 153-154.
22. VeIdstra, H., "Research on plant growth-regulators. XXI. Halogenated benzoic acids and related compounds," Rec. trav. chim. 71, 151 (1952).

23. Wain, R. L., and Fawcett, C. H., in Plant Physiology: A Treatise. Volume VA: Analysis of Growth: Behavior of Plants and Their Organs (F.C. Steward, Ed.) New York and London, Academic Press, Inc., 1969, pp. 231-296.
24. Wain, R. L., and Sightman, F., "Studies on plant growth-regulating substances. VII. Growth-promoting activity in the chlorophenoxyacetic acids," Ann. Appl. Biol. 40, 244-249 (1953).
25. Wohl, A., "Über estersäuren und amidsäure der isophthal-säurereihe, ein beiträg zur frage de gleichwertigkeit der stellungen 2 und 6 am benzolkern," Berichte 43, 3474-3489 (1910).
26. Zanger, M., "The determination of aromatic substitution patterns by nuclear magnetic resonance," Organic Magnetic Resonance 4, 1-25 (1972).
27. Zimmerman, P. W., and Hitchcock, A. E., "Substituted phenoxy and benzoic acid growth substances and the relation of structure to physiological activity," Contrib. Boyce Thompson Inst. 12, 321-343 (1942).