

# Relation Between Molecular Structure and Physiological Activity of Plant Growth Regulators

## II. Formative Activity of Phenoxyacetic Acids

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The formative activities of approximately 145 ring-substituted phenoxyacetic acids have been measured by the bean leaf repression technique. The presence of a halogen atom at position 4 appears to be a requisite for high activity. The order of effectiveness of the halogens is chlorine > fluorine > bromine > iodine. Further enhancement of activity may ensue through introduction of an additional halogen or methyl substituent at position 2.

A LARGE NUMBER of substituted benzoic and aryloxyalkanoic acids, as well as certain of their derivatives, have the capability to modify profoundly the size, shape, and structure of developing plant organs (5). This property, which has been designated "formative activity" (70), appears in general to be correlated with herbicidal potency of such compounds. For the determination of the structural requisites for formative activity and their ultimate interpretation in terms of action mechanisms, there are needed comparative, quantitative data which have not hitherto been available. The present report provides such data for a number of ring-substituted phenoxyacetic acids. Subsequent papers will deal with certain functional derivatives, homologs, and analogs of the phenoxyacetic acids and other series of compounds.

### Methods

Although direct quantitative measurement of formative responses offers considerable difficulty, the leaf repression method of Brown and Weintraub (7) provides a convenient and fairly precise bioassay for formative activity. This assay is carried out by applying known amounts of the test compound to buds of bean plants of carefully standardized size and determining the subsequent repression of leaf expansion which accompanies the characteristic formative modification. The method was designed to minimize the influence of penetrability and transportability of the test compounds, so as to approximate as closely as possible the "intrinsic" activity. Activities are measured in absolute units, the molar leaf area repression activity (MOLARA) being defined as the reciprocal of the dose in micromoles which results in a 50% repression of area as compared with an untreated control leaf. Repeated retests of several compounds

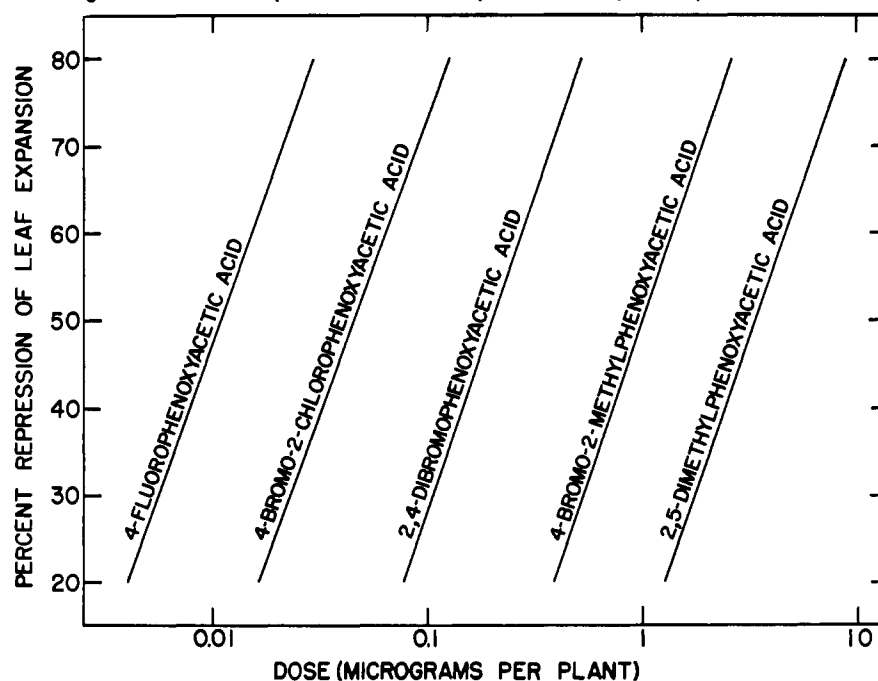
during a 6-year period have demonstrated that the method has a high degree of reproducibility.

In practice the dose-response curve over the range 20 to 80% repression was determined for each compound by testing a graded series of doses; the curves were calculated by least squares. This method permits the evaluation with equal precision of compounds which differ in activity by several orders of magnitude. For many substances of very low activity, however, it is possible only to indicate a maximal limit, inasmuch as the excessively high doses that must be applied to the plant may result in a non-formative type of injury to the young leaf. Although these limiting values have been indicated in the tables, such

compounds may be regarded as inactive under the conditions of the bioassay.

Each of the dose-response curves from which the present values have been calculated was determined from at least two, usually more, separate experiments, each comprising three or more doses with 16 replicate plants per dose; the evaluation of each compound required, on the average, about 200 plants. The mean 95% confidence interval, as determined from a sampling of 40 compounds of moderate to high activity, was  $\pm 16\%$  of the molar leaf area repression activity. With few exceptions, the slopes of the curves for all the active compounds are nearly the same, giving support to the view that a common mechanism of action is involved. Some examples are shown in Figure 1.

Figure 1. Dose-response curves for representative phenoxyacetic acids



**Table I. Formative Activities of Monosubstituted Phenoxyacetic Acids**

Substituent		Position		
		2	3	4
Acetamido	CH <sub>3</sub> CO.NH—	...	<10	...
Acetyl	CH <sub>3</sub> CO—	...	...	<10
Amino	NH <sub>2</sub> —	<17	<17	<17
Benzoyl	C <sub>6</sub> H <sub>5</sub> CO—	...	...	<13
Bromine	Br—	<23	<116	6,160
<i>tert</i> -Butyl	(CH <sub>3</sub> ) <sub>3</sub> C—	...	...	<21
Carboxyl	HOOC—	<10	<10	<10
Chlorine	Cl—	<19	<37	18,700
<i>o</i> -Chlorophenyl	<i>o</i> -ClC <sub>6</sub> H <sub>4</sub> —	<26	...	...
<i>p</i> -Chlorophenyl	<i>p</i> -ClC <sub>6</sub> H <sub>4</sub> —	<52	...	...
Ethoxyl	C <sub>2</sub> H <sub>5</sub> O—	...	...	<10
Ethyl	C <sub>2</sub> H <sub>5</sub> —	<18	<18	<18
Fluorine	F—	<8	<8	15,500
Formyl	HCO—	<9	...	<9
Hydroxyl	HO—	<17	...	<8
Iodine	I—	<28	...	443
Methoxyl	CH <sub>3</sub> O—	<18	184	32
Methyl	CH <sub>3</sub> —	<17	19	30
Nitro	O <sub>2</sub> N—	<10	<20	<10
Nitroso	O:N—	...	...	<9
Oximidomethyl	HON:CH—	<10	...	21
Oxyacetic	HOOC.CH <sub>2</sub> O—	<11	<11	...
Phenyl	C <sub>6</sub> H <sub>5</sub> —	...	...	<11
<i>n</i> -Propyl	CH <sub>3</sub> CH <sub>2</sub> CH <sub>2</sub> —	...	...	<19
Thiocyano	NCS—	...	...	<40
Trifluoromethyl	CF <sub>3</sub> —	...	<23	...

**Results**

Phenoxyacetic acid itself shows no activity (MOLARA < 11).

**Monosubstituted Phenoxyacetic Acids.** Results for all the monosubstituted compounds tested are summarized in Table I. Active compounds are obtained by introduction into a suitable position of the ring of a single halogen, methyl, methoxyl, or oximidomethyl substituent, but not by any of the other groups listed.

None of the investigated substituents confers activity when in the ortho position. A halogen atom is activating only when present in the para position. On the other hand, both the meta and para positions are suitable for a methyl or methoxyl group; in the case of the latter the meta position is considerably more favorable than the para.

Chlorine is the most potent of the halogens, followed by fluorine, bromine, and iodine; the relative effectiveness is 1.0, 0.8, 0.3, and 0.02, respectively.

**Disubstituted Phenoxyacetic Acids.** Introduction of a second ring substituent into an active 4-halogenophenoxyacetic acid may enhance, decrease, or not affect the activity, depending upon the particular substituents involved (Table II).

In the 4-chloro series, introduction into position 2 of any substituent, except another chlorine atom, results in great diminution of activity. The 2,4-dichloro compound, however, is somewhat more active than the 4-chloro acid.

On the other hand, a methyl substituent in position 2 markedly augments the activity of 4-fluorophenoxyacetic acid. The potency of the 4-bromo and 4-iodo compounds also is increased by introduction of a suitable halogen atom into position 2.

Additional substitution into the inactive 2- or 3-chlorophenoxyacetic acids may enhance activity, as shown by the 2,6- and 2,3-dichloro compounds (Table III).

Similarly, the introduction of a second substituent into a nonhalogenated phenoxyacetic acid may increase the activity. Unfortunately, data are available for only two series of compounds, the activities of several of which are below the detectable limit, so that the full extent of the augmentation cannot be determined. Nevertheless, 2,5- and 3,4-dimethylphenoxyacetic acids (Table III) are appreciably more active than any of the monomethyl compounds and 2,4-dinitrophenoxyacetic acid (Table IV) is more active than either the 2-nitro or 4-nitro compound.

In addition to these, two other active compounds with a nonhalogen substituent at position 4 have been encountered: 2-chloro-4-carboxymethoxyacetic and 2,4,5-trimethylphenoxyacetic acids, both of low potency.

**Trisubstituted Phenoxyacetic Acids.** Activities of the 22 compounds that have been tested are presented in Table V. The activity of 2,4-dichlorophenoxyacetic acid is diminished by introduction of a

third chlorine atom in any position; the inactivating effect of the third substituent is least at position 3 and greatest at position 6. 3,4,5-Trichlorophenoxyacetic acid has approximately the same activity as the 3,4-dichloro derivative. 2,3,6-Trichlorophenoxyacetic acid is appreciably more active than either the 2,3- or 2,6-dichloro compound. A methyl or carboxyl group at position 6 reduces the activity of 2,4-dichlorophenoxyacetic acid below the measurable level. In contrast to the 2,4,6-trichloro compound, 2,4,6-trifluorophenoxyacetic acid exhibits moderately high activity.

2,4,5-Trichlorophenoxyacetic and 5-bromo-2,4-dichlorophenoxyacetic acids, while exhibiting relatively high potencies in suppressing leaf expansion, do not evoke the typical morphological response brought about by all the other active compounds reported in this paper.

As in the case of the disubstituted compounds, methyl groups appear to exert a very different influence than the halogens. Thus, 2,4,5-trimethylphenoxyacetic acid is somewhat more active than any of the dimethyl derivatives, whereas 3,4,5-trimethylphenoxyacetic acid is inactive.

**Tetra- and Pentasubstituted Phenoxyacetic Acids.** The following compounds were tested:

- 2-Methyl-4,*x,y*-trichlorophenoxyacetic acid, MOLARA < 54
- 2,4-Dichloro-3,5-dimethylphenoxyacetic acid, MOLARA = 590

**Table II. Influence of Second Substituent in Position 2 on Formative Activities of 4-Halogenophenoxyacetic Acids**

Substituent in Position		
2	4	MOLARA
...	F	15,500
CH <sub>3</sub>	F	25,200
F	F	4,370
I	F	1,220
...	Cl	18,700
F	Cl	5,660
Cl	Cl	21,900
Br	Cl	4,120
I	Cl	1,410
CH <sub>3</sub>	Cl	749
CH <sub>2</sub> .CH <sub>3</sub>	Cl	464
CH <sub>2</sub> .CH <sub>2</sub> .CH <sub>3</sub>	Cl	104
CH(CH <sub>3</sub> ).CH <sub>2</sub> .CH <sub>3</sub>	Cl	<23
CH <sub>2</sub> .CH <sub>2</sub> .CH <sub>2</sub> .CH <sub>2</sub> .CH <sub>3</sub>	Cl	<24
CH <sub>2</sub> .CH:CH <sub>2</sub>	Cl	<45
CH.CH <sub>2</sub> .CH <sub>2</sub> .CH <sub>2</sub> .CH <sub>2</sub> .CH <sub>2</sub>	Cl	<27
CH <sub>2</sub> .C <sub>6</sub> H <sub>5</sub>	Cl	<28
CH <sub>2</sub> Cl	Cl	124
CH <sub>2</sub> .CHCl.CH <sub>2</sub> Cl	Cl	<30
CH <sub>2</sub> CN	Cl	469
COOH	Cl	<23
CH <sub>2</sub> .COOH	Cl	<12
CO.CH <sub>3</sub>	Cl	<23
OH	Cl	267
OCH <sub>3</sub>	Cl	<40
NO <sub>2</sub>	Cl	36
...	Br	6,160
F	Br	1,570
Cl	Br	5,920
Br	Br	11,500
CH <sub>3</sub>	Br	345
CHO	Br	197
CH:NOH	Br	117
...	I	443
F	I	122
Cl	I	1,880
I	I	344

Pentachlorophenoxyacetic acid,  
MOLARA < 61

### Discussion

Inasmuch as there are a considerable number of very diverse plant growth responses to chemicals, and the relative activities of various compounds are not alike in inducing these different types of response, the structure-activity relationships cannot necessarily be extrapolated from one type of response to another (5,9). The activity values here reported apply strictly only to the formative response in beans as measured by the specific technique employed. However, experience at this laboratory indicates that there is a relationship between formative activity measured in this way and herbicidal activity against a number of plant species. This statement is intended only as a broad generalization, to which many exceptions are already known; without

doubt, many more examples of pronounced specificities remain to be discovered.

Although the present data are in general agreement with the semiquantitative results of earlier studies of herbicidal and formative activities of substituted phenoxyacetic acids (3; citations in 5), there are a few discrepancies, due possibly to differences in the test plants employed—for example, 2-chlorophenoxyacetic acid was reported to have high formative activity in tomato (77) but was found inactive in the present study.

The problem of the relation between formative activity and auxin activity is difficult to resolve at present, in large part because of the paucity of data that can be used for comparison. While a number of compounds with high formative activity, such as 2,4-dichlorophenoxyacetic acid, also possess high

auxin activity, others have little or none and, conversely, certain of the most potent auxins are inactive as formagens. Indeed, in its formative action, 2,4-dichlorophenoxyacetic acid appears to behave as an antiauxin (7).

An interesting example of the differences in structural requirements for different types of growth-regulatory activity is to be found in relation to ortho-substitution in the phenoxyacetic acids. Tests with a bioassay depending upon stimulation of cell elongation have led to the hypothesis that growth-regulatory activity in this series of compounds is dependent upon the presence of an unsubstituted position ortho to the oxyacetic group (2, 4). The present results give further evidence that such a generalization does not apply to the formative response, inasmuch as formative activity is exhibited by 2,4,6-trifluoro-, 2,3,6-trichloro-, 2,6-dichloro-, and 2,6-difluorophenoxyacetic acids; 2,4,6-trichlorophenoxyacetic acid was reported by Zimmerman (77) to produce formative effects on tomato. A free ortho-position may not be essential even for all types of auxin activity; Wain (6) has reported that 2,4-dichloro-6-fluorophenoxyacetic and 2,4-dibromo-6-fluorophenoxyacetic acids show high activity in the Went pea test.

The significance of the structure-activity relationships will be discussed in a later paper of this series.

### Acknowledgment

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**Table III. Formative Activities of Dichloro-, Difluoro-, and Dimethylphenoxyacetic Acids**

Substituted Positions	MOLARA		
	Dichloro	Dimethyl	Difluoro
2,4	21,900	<36	4,370
3,4	1,210	41	...
2,6	135	...	...
2,3	85	<36	...
2,5	<69	54	21
3,5	<44	<18	32

The majority of the compounds tested were synthesized either at Camp Detrick under the direction of H. E. Thompson or of W. L. Howard, or at Ohio State University under the direction of M. S. Newman. A few were obtained from commercial sources and through the Chemical-Biological Coordination Center of the National Research Council. For the remainder the authors are indebted to F. H. Reed and G. C. Finger

Table IV. Formative Activities of Disubstituted Phenoxyacetic Acids

Substituent in Position						MOLARA
2	3	4	5	6		
F	...	CH <sub>3</sub>	...	...	<18	
Cl	...	CH <sub>3</sub>	...	...	<20	
Br	...	CH <sub>3</sub>	...	...	<12	
OCH <sub>3</sub>	...	CH <sub>3</sub>	...	...	<10	
NO <sub>2</sub>	...	NO <sub>2</sub>	...	...	14	
Cl	...	NO <sub>2</sub>	...	...	<23	
Cl	...	OCH <sub>2</sub> COOH	...	...	7	
Br	...	C <sub>6</sub> H <sub>5</sub>	...	...	<31	
Cl	...	C(CH <sub>3</sub> ) <sub>3</sub>	...	...	<23	
Br	...	C(CH <sub>3</sub> ) <sub>3</sub>	...	...	<19	
CH <sub>2</sub> (CH <sub>3</sub> ) <sub>2</sub>	...	...	CH <sub>3</sub>	...	<42	
...	CH <sub>3</sub>	F	...	...	152	
...	CH <sub>3</sub>	Cl	...	...	502	
...	Br	...	CH <sub>3</sub>	...	<24	
...	C <sub>2</sub> H <sub>5</sub>	...	CH <sub>3</sub>	...	<40	
...	Br	...	Br	...	<62	
F	...	...	...	F	19	
Cl	...	...	...	CH <sub>3</sub>	<40	
COOH	...	...	...	CH <sub>3</sub>	<10	

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Table V. Formative Activities of Trisubstituted Phenoxyacetic Acids

Substituent in Position						MOLARA
2	3	4	5	6		
F	...	F	F	...	10,200	
F	...	F	...	F	1,610	
Cl	Cl	Cl	...	...	14,200	
Cl	Cl	...	...	Cl	398	
Cl	...	Cl	Cl	...	<4,740	
Cl	...	Cl	Br	...	<3,000	
Cl	...	Cl	OH	...	<12	
Cl	...	Cl	...	Cl	<25	
Cl	...	Cl	...	CH <sub>3</sub>	<78	
Cl	...	Cl	...	COOH	<13	
...	Cl	Cl	Cl	...	1,610	
...	CH <sub>3</sub>	Cl	CH <sub>3</sub>	...	555	
COOH	...	Cl	...	CH <sub>3</sub>	<49	
Br	...	Br	...	CH <sub>3</sub>	<65	
COOH	...	Br	...	CH <sub>3</sub>	<29	
I	...	I	...	I	<26	
Cl	...	CH <sub>3</sub>	...	Cl	<23	
CH <sub>3</sub>	CH <sub>3</sub>	...	CH <sub>3</sub>	...	<19	
CH <sub>3</sub>	...	CH <sub>3</sub>	CH <sub>3</sub>	...	98	
...	CH <sub>3</sub>	CH <sub>3</sub>	CH <sub>3</sub>	...	<19	
Cl	NO <sub>2</sub>	...	...	Cl	<27	
C(CH <sub>3</sub> ) <sub>3</sub>	...	C(CH <sub>3</sub> ) <sub>3</sub>	CH <sub>3</sub>	...	<14	