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Structure-Activity Relationship in the Auxin Activity of Mono-Substituted Phenylacetic Acids[1]

Robert M. Muir, Toshio Fujita[2], and Corwin Hansch
Department of Botany, University of Iowa, Iowa City, Iowa 52240
and
Department of Chemistry, Pomona College, Claremont, California

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Summary. The analysis of substituent constants for the lipophilic and electronic factors in the auxin activity of substituted phenylacetic acids in elongation of coleoptile segments shows that these factors parallel those for the phenoxyacetic acids but assign reactivity in growth promotion to the meta position of phenylacetic acid. The inhibitory effects with supra-optimal concentrations are highly dependent on the lipophilic character of the molecules.

The auxin activity of phenylacetic acid has been known for a long time (6, 25) and has merited considerable study (2, 23, 24) but only 1 comparison of the effects of single ring substituents has been published (19). In this study Melnikov et al. (19) observed that halogen substitution in the ring increased auxin activity while methyl substitution decreased auxin activity, and substitution in the 3- or 4-positions of phenylacetic acid had opposite effects to substituents in the 3- or 4-positions of phenoxyacetic acid. These observations were based on only a few substituents and an incomplete series so their significance has been somewhat limited.

Our success in relating structure and activity in mono-ring-substituted phenoxyacetic acids by the use of substituent constants (9, 10) suggested that a similar analysis should be applied to phenylacetic acids. The analysis is based on the hypothesis of a 2-point reaction of the growth regulator with the plant substrate, first through the carboxyl group and then at a position on the aromatic ring (20). Initially the evidence indicated that the position on the ring ortho to the side chain carrying the carboxyl group was the site of the second reaction, but later the experimental evidence (21) required that the position of attachment on the ring would depend on the particular combination of steric and electronic factors. It is now clear (10) that a substituent in a particular biologically active molecule may induce important changes of a steric, electronic and lipophilic character.

Very little information can be assembled on the steric factors in auxin activity; empirically it is apparent that functions of modest size in the 3-position and very small functions in the 4-position have little steric effect. The electronic effect can be measured by the use of the substituent constant, σ, which has been developed for the benzene ring (15). The lipophilic effect of the substituent can be evaluated by the use of a new substituent constant, π, for hydrophobic bonding (5, 11, 12, 14). π is a measure of the lipophilic-hydrophilic character of the substituent and is defined as the logarithm of the partition coefficient between *l*-octanol and water of a derivative minus the logarithm of the partition coefficient of the parent compound. The electronic effect of a substituent is distinguished from its lipophilic effect as being a small change in electron density which would affect the rate or equilibrium constant of a highly specific chemical action or charge-transfer complex, but would not make a significant difference in π.

Materials and Methods

Promotive and inhibitive effects on elongation were determined with segments of *Avena* coleoptile tissue 3 mm in length. The oats of the cultivar Victory were grown in sand in total darkness at 24° and 85 % relative humidity. When the coleoptile was 2 to 2.5 cm long, the apical 2 mm were removed and 2 hours later an apical segment was excised. All manipulations were carried out under red light. The segments were then floated on 25 ml of solution in a covered Petri dish in darkness. The solution containing the growth regulator also contained 2.5 mM potassium maleate at pH 4.5 and 0.09 M sucrose since these constituents have been shown to maintain constant growth rates (18). After 24 hours the length of the segment was measured to the nearest 0.05 mm under the binocular microscope. In comparing the auxin activity of the phenylacetic acids, the concen-

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[2] Permanent address: Department of Agricultural Chemistry, Kyoto University, Kyoto, Japan.

tration of the substituted compound was determined which induced the same amount of elongation as did phenylacetic acid at 10 μM which was usually 10 % over the control. The inhibitory effect of the growth regulators was compared by determining the concentration which reduced elongation by 5 % below the control.

The phenylacetic and phenoxyacetic acids used in this investigation were synthesized by methods which have been reported except for 3-*n*-propylphenyl-acetic acid. This compound was prepared by adding 200 mg of sulfur to 1.1 g of 3-*n*-propylacetophenone (3) in 6 ml of morpholine. The mixture was refluxed for 12 hours on an oil bath at 120° after which time 5 ml of glacial acetic acid and 10 ml of 48 % hydrobromic acid were added and refluxing was continued for 4 hours. Most of the acetic acid was then removed by vacuum evaporation and the residue was diluted with water and extracted with ether. The ether was washed with water and extracted with sodium bicarbonate solution. The bicarbonate layer was then acidified and extracted with ether. The residue from evaporation of the ether was vacuum-distilled. Yield: 0.4 g, b.p. 132 to 133°/2 mm. After recrystallization from pentane the product melted at 53.5 to 54.5°. Analysis calculated for $C_{11}H_{14}O_2$: C, 74.13; H, 7.92. Found: C, 73.99; H, 7.92.

The values assigned to π in this study are based on measurements of the partition coefficients of phenylacetic acid (PAA) and phenoxyacetic acid (POA) and their derivatives between purified *l*-octanol and distilled water. The C, H and O ratio of octanol is approximately that of the natural oleates. The partition coefficient is calculated as

$$P = \frac{\text{Coctanol}}{C_{H_2O}\ (1-\alpha)},$$

where α is the degree of dissociation of the acid in the water phase (10) and the partition coefficient refers to the unassociated and non-ionized acid. The determination of the concentration in the water phase was made coulometrically (10) or spectrophotometrically (5). The concentration in the octanol phase was found by difference.

All solutions of the growth regulators were prepared in water (glass-distilled) heated to boiling and cooled just before their auxin activity was assayed. The pH of the solutions was adjusted to 4.5 with NaOH.

Results and Discussion

The auxin activity of phenylacetic acid in the elongation of subapical segments of *Avena* coleoptiles is shown in figure 1. The values plotted are the averages of 3 trials with tissue from plants grown at different times. The activity is typically that of a weak auxin with a limited effect over a small range of concentrations.

The concentrations of the mono-substituted phenyl-

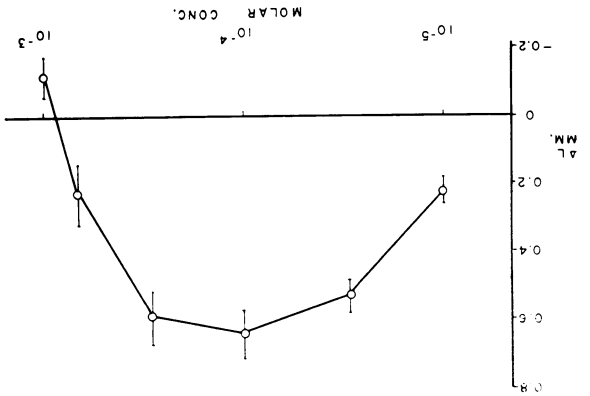
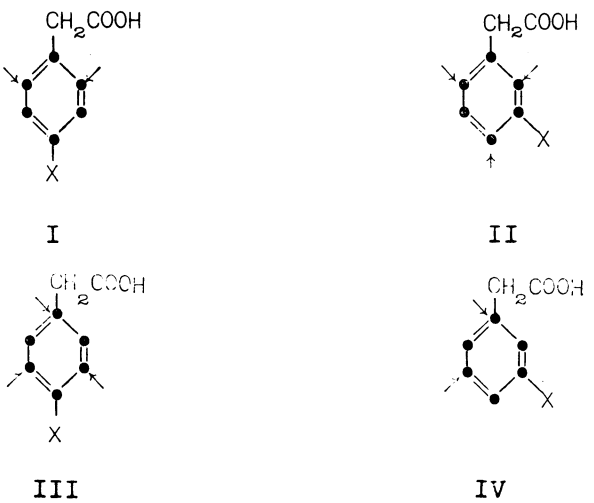


FIG. 1. Effect of phenylacetic acid on the growth of subapical segments of *Avena* coleoptiles.

acetic acids inducing an elongation greater by 10 % than the percent elongation taking place in the controls are given in table I along with the parameters for substituent effects on relative electron density on the ring, σ_1 and σ_2, and the lipophilichydrophilic character of the substituent, π. The electronic effect of the substituent, X, on the ortho position in determining the relative auxin activity is represented by σ_1. Here, as shown below for I, σ_1 is σ_m for a substituent in the 4-position, and as shown for II, σ_1 is σ_p for a substituent in the 3-position. The arrows indicate the positions for which σ is a measure of the relative electron density (15). It is assumed that the substituent affects the positions ortho to it in much the same way that it affects the position para to it. For σ_2 the electronic effect of the substituent, X, is greatest on the meta position and the position of attachment of the side chain. As shown for III, σ_2 is σ_p for a substituent in the 4-position and, as shown for IV, σ_2 is σ_m for a substituent in the 3-position.



Of course, electronic effects of the substituents change the electron density of all of the atoms in the molecule. However, short of quantum mechanical

calculations of relative electron densities for each atom (and it is unlikely that such calculations for molecules as complex as those in table I would yield results superior to those found with σ^-), σ seems to be the best parameter for relative electronic effects now available. The arrows in I to IV simply indicate the points of greatest electronic effect of X for each of the 2 possibilities of reaction on the ring.

By means of regression analysis it is possible to make a quantitative analysis of substituent effects on the biological activity of the series of growth regulators and to compare the importance of electron density as represented by σ_1 at the positions ortho to the position of attachment of the side chain with that represented by σ_2 at the positions meta to the side chain. The following equations were derived from the data of table I by the method of least squares using an IBM 360/40 computer. The number of points used in the regression analysis is represented by n, r is the multiple correlation coefficient and s is the standard deviation.

The higher correlations and lower standard deviations for equations IV and VIII indicate that these fit the data best in terms of σ and π. Equations V and IX were derived to check the possibility that the dependence of activity on σ might not be linear. In neither instance does the inclusion of the σ^2 term give an improved correlation. (Compare values of s with those of equations IV and VIII). The 2 best equations are equations IV and VIII in which there is included the interaction term, $\pi\sigma$, a refinement in the method which has recently been investigated (13). The considerable reduction in the variance obtained with equation VIII over that with equation IV indicates that a decrease in electron density (positive coefficient with σ) at the meta position of phenylacetic acid is more important for greater biological activity than a decrease at the ortho position. We have also investigated the use of the parameters σ^- and σ^- (16) in equations of the above type and found that these do not give as good correlations as σ.

For the substituted phenoxyacetic acids in which the decrease in electron density at the ortho position appears to be more important (10), the equations with electron density as represented by σ_1 and σ_2 corresponding to equation IV and VIII respectively are:

Equations derived with σ_1:

	n	r	s	
$\log \frac{1}{C} = 0.930\pi + 5.405$	16	0.690	0.637	(I)
$\log \frac{1}{C} = 0.919\pi + 0.935\sigma + 5.256$	16	0.784	0.568	(II)
$\log \frac{1}{C} = -0.750\pi^2 + 1.528\pi + 0.836\sigma + 5.433$	16	0.845	0.509	(III)
$\log \frac{1}{C} = -0.835\pi^2 + 1.404\pi + 0.672\sigma + 1.474(\pi\sigma) + 5.456$	16	0.908	0.416	(IV)
$\log \frac{1}{C} = -0.851\pi^2 + 1.439\pi + 0.336\sigma^2 + 0.532\sigma + 1.479(\pi\sigma) + 5.430$	16	0.909	0.435	(V)

Equations derived with σ_2:

	n	r	s	
$\log \frac{1}{C} = 0.832\pi + 1.390\sigma + 5.140$	16	0.829	0.512	(VI)
$\log \frac{1}{C} = -0.557\pi^2 + 1.300\pi + 1.161\sigma + 5.304$	16	0.858	0.488	(VII)
$\log \frac{1}{C} = -0.703\pi^2 + 1.084\pi + 1.122\sigma + 2.020(\pi\sigma) + 5.259$	16	0.941	0.337	(VIII)
$\log \frac{1}{C} = -0.812\pi^2 + 1.249\pi + 0.948\sigma^2 + 0.652\sigma + 1.996(\pi\sigma) + 5.245$	16	0.945	0.341	(IX)

	n	r	s	
$\log \frac{1}{C} = -1.977\pi^2 + 3.242\pi + 1.865\sigma + 4.162$	21	0.881	0.484	(X)
$\log \frac{1}{C} = -1.548\pi^2 + 2.665\pi + 1.466\sigma + 4.277$	21	0.795	0.633	(XI)

Table I. *Correlation of Structure of Phenylacetic Acids with Activity in Promoting Elongation*

Function	σ_1	σ_2*	π**	Molar conen for +10 % effect	Obsd $\log \frac{1}{C}$	Calcd*** $\log \frac{1}{C}$
3-I	0.28	0.35	1.22	5×10^{-8}	7.3	6.79
3-CF_3	0.55	0.42	1.16	2×10^{-7}	6.7	7.03
3-Br	0.23	0.39	0.91	2×10^{-7}	6.7	6.82
3-Cl	0.23	0.37	0.68	3×10^{-7}	6.5	6.59
3-SCH_3	—0.05	0.14	0.62	4×10^{-7}	6.4	5.99
3-NO_2	0.78	0.71	—0.01	7×10^{-7}	6.2	6.03
3-CH_3	—0.17	—0.07	0.49	2×10^{-6}	5.7	5.47
4-F	0.34	0.06	0.14	2×10^{-6}	5.7	5.48
3-F	0.06	0.34	0.19	3×10^{-6}	5.5	5.95
3-CN	0.63	0.68	—0.28	3×10^{-6}	5.5	5.28
3-OCH_3	—0.27	0.12	0.04	5×10^{-6}	5.3	5.45
H	0.00	0.00	0.00	10^{-5}	5.0	5.26
3-$COCH_3$	0.52	0.31	—0.28	2×10^{-5}	4.7	5.07
3-OH	—0.36	0.00	—0.52	2×10^{-5}	4.7	4.51
4-OH	0.00	—0.36	—0.61	4×10^{-5}	4.4	4.38
3-n-Pr	—0.12	—0.04	1.43	10^{-5}	5.0	5.21

* From reference 15.
** From reference 5.
*** Calculated using equation VIII.

Addition of $(\pi\sigma)$ and σ^2 terms to equations X and XI did not yield equations giving better correlations. The significance of the $(\pi\sigma)$ term in equations IV and VIII and its lack of significance for equations X and XI is not immediately apparent and warrants further study. Equation X accounts for about 15 % more of the variance in the data than does equation XI and indicates that the model of electronic effects shown in (1) and (2) for σ_1 best represents the phenoxyacetic acid series. In the 2-point reaction mechanism the meta position of the phenylacetic acid molecule could be the favored reaction site because of the molecular geometry. With 1 less atom in the side chain, the ring formed by a 2-point reaction involving the meta position of phenylacetic acid would be more similar to the ring formed at the ortho position of phenoxyacetic acid.

The coefficients associated with σ in equations II to IX are, in general, smaller than those in equations X and XI, indicating a greater dependence of biological activity on electron withdrawal in the POA series. This is probably caused in part by the electron-releasing effect of the ether oxygen linkage. The lower electron-releasing effect of the acetic acid side chain in PAA would thus account in part for the auxin activity of PAA being 30 times that of POA. Of course, the geometry of the 2-point attachment of the 2 systems must also be considered.

One of the characteristic properties of the auxin molecule which has been recognized for a long time is its capacity to inhibit, as well as promote, elongation. An optimal concentration exists for the promotive effect and at higher concentrations lesser elongation takes place. With increasing concentrations a point is reached where the auxin will inhibit the elongation taking place in tissue. The inhibition of elongation by auxins has been examined both experimentally (1, 2) and theoretically (4) with the latter treatment being based on a 2-point reaction mechanism. In our earlier study of the roles of σ and π in the POA derivatives promoting elongation, we found that for the more lipophilic molecules the inhibitory effect appeared at concentrations well below those predicted to promote elongation (10). In the PAA series the 3-*n*-propyl derivative, the most lipophilic analog, has lesser promotive effects than many hydrophilic analogs (table I). These observations suggest that inhibition develops with accumulation at the site of reaction causing ultrastructure aberrations. The possibility that a change in ultrastructure is responsible for the transition from promotive to inhibitory effects is currently under investigation.

The inhibitory action of auxins of the POA series was analyzed for dependence on electron density at the ortho position and π using concentrations which give elongation 5 % less than the control tissue (C_i). The regression analysis showed no dependence on electron density in the ring. The substituent effect on the side chain was then examined and the data are given in table II with values of σ referring to the position of attachment of the side chain rather than the ortho position and values of π as recently determined (5). Equations XII and XIII were derived from the data in table II.

$$\downarrow$$

	n	r	s	
$\log \frac{1}{C_i} = 0.778\pi + 2.912$	22	0.928	0.225	(XII)
$\log \frac{1}{C_i} = 0.800\pi + 0.222\sigma + 2.845$	22	0.933	0.223	(XIII)

Table II. *Correlation of Structure of Phenoxyacetic Acids with Activity in Inhibiting Elongation*

Function	σ*	π*	Molar concn for −5 % effect	Obsd $\log \frac{1}{C_i}$	Calcd** $\log \frac{1}{C_i}$
3-C_6H_5	0.22	1.89	6×10^{-5}	4.2	4.38
3-n-Bu	−0.04	1.90	7×10^{-5}	4.1	4.39
3-I	0.35	1.15	10^{-4}	4.0	3.81
3-n-Pr	−0.04	1.43	1.1×10^{-4}	3.9	4.02
3-Br	0.39	0.94	1.5×10^{-4}	3.8	3.64
3-CF_3	0.42	1.07	2.0×10^{-4}	3.7	3.74
3-Cl	0.37	0.76	2.5×10^{-4}	3.6	3.50
3-Et	−0.04	0.97	3.2×10^{-4}	3.5	3.67
3-CH_3	−0.07	0.51	5×10^{-4}	3.3	3.31
3-F	0.34	0.13	7×10^{-4}	3.2	3.01
3-OCH_3	0.12	0.12	8×10^{-4}	3.1	3.01
H	0.00	0.00	10^{-3}	3.0	2.91
3-NO_2	0.71	0.11	1.4×10^{-3}	2.9	3.00
3-$COCH_3$	0.31	−0.28	2.5×10^{-3}	2.6	2.69
3-CN	0.63	−0.30	3.3×10^{-3}	2.5	2.68
3-OH	0.00	−0.49	10^{-2}	2.0	2.53
4-I	0.28	1.26	5×10^{-5}	4.3	3.89
4-Cl	0.23	0.70	2×10^{-4}	3.7	3.46
4-CH_3	−0.17	0.52	6×10^{-4}	3.2	3.32
4-F	0.06	0.15	7×10^{-4}	3.1	3.03
4-$COCH_3$	0.52	−0.37	10^{-3}	3.0	2.62
4-NO_2	0.78	0.24	10^{-3}	3.0	3.10

* See table I.
** Calculated using equation XII.

Equation XII shows that the inhibitory effect depends entirely on the lipophilic character of the substituent; the larger the value of π, the lower the concentration required for inhibition. The slight reduction in variance obtained with equation XIII is not significant.

Since the promotion of elongation by the PAA series was found to be dependent on a decrease in electron density at the meta position, the inhibitory activity was also examined for dependence on this electron effect and π. The data are given in table III. The slight differences in concentration of the auxin causing promotion and inhibition of elongation are well illustrated by 3-iodophenylacetic acid which at 1.5×10^{-4} M gives elongation that is 12 % over the control, while at 1.7×10^{-4} M reduces elongation to 5 % less than the control.

Equations XIV and XV were derived from the data of table III.

$$\log \frac{1}{C_i} = 0.584\pi + 3.166 \qquad n = 20,\ r = 0.761,\ s = 0.315 \qquad \text{(XIV)}$$

$$\log \frac{1}{C_i} = 0.562\pi + 0.624\sigma + 3.038 \qquad n = 20,\ r = 0.872,\ s = 0.245 \qquad \text{(XV)}$$

An F test shows the additional term in equation XV to be significant at >0.99 level of significance. Even though the correlation is improved by the inclusion of the σ term in equation XV, it is still not nearly as good as the correlation obtained with equation XII for the POA series without the σ term. An inspection of the data shows that 2 points are very poorly fit, the 4-NO_2 and the 4-OCH_3. Omitting these 2 points, we obtain equations XVI and XVII.

$$\log \frac{1}{C_i} = 0.731\pi + 3.017 \qquad n = 18,\ r = 0.971,\ s = 0.114 \qquad \text{(XVI)}$$

$$\log \frac{1}{C_i} = 0.705\pi + 0.234\sigma + 2.988 \qquad n = 18,\ r = 0.983,\ s = 0.092 \qquad \text{(XVII)}$$

Although the additional term in equation XVII is statistically significant ($F_{1,15} = 8.92$) with the set of derivatives in hand, only a very small improvement in correlation is obtained.

Table III. *Correlation of Structure of Phenylacetic Acids with Activity in Inhibiting Elongation*

Function	σ*	π*	Molar concn for —5% effect	Obsd $\log \frac{1}{C_i}$	Calcd** $\log \frac{1}{C_i}$
3-n-Pr	—0.04	1.43	10^{-4}	4.0	4.06
3-CF_3	0.42	1.16	10^{-4}	4.0	3.87
3-I	0.35	1.22	1.7×10^{-4}	3.8	3.91
3-Br	0.39	0.91	2×10^{-4}	3.7	3.68
3-Cl	0.37	0.68	3.1×10^{-4}	3.5	3.52
3-CH_3	—0.07	0.49	5×10^{-4}	3.3	3.38
3-NO_2	0.71	—0.01	5×10^{-4}	3.3	3.01
3-F	0.34	0.19	7×10^{-4}	3.2	3.16
H	0.00	0.00	10^{-3}	3.0	3.02
3-CN	0.68	—0.28	1.25×10^{-3}	2.9	2.81
3-OCH_3	0.12	0.04	1.3×10^{-3}	2.9	3.05
3-OH	0.00	—0.52	3×10^{-3}	2.5	2.64
4-NO_2	0.78	—0.04	10^{-4}	4.0	3.92
4-I	0.28	1.23	10^{-4}	4.0	2.99
4-$COCH_3$	0.52	—0.37	2×10^{-4}	3.7	2.75
4-Cl	0.23	0.70	3×10^{-4}	3.5	3.53
4-CH_3	—0.17	0.45	5×10^{-4}	3.3	3.35
4-F	0.06	0.14	6×10^{-4}	3.2	3.12
4-OCH_3	—0.27	0.01	1.1×10^{-3}	2.9	3.03
4-OH	—0.36	—0.61	2.2×10^{-3}	2.6	2.57

* See table I.
** Calculated using equation XVI.

The studies of both promotive effects and inhibitive effects show clearly that while POA and PAA have a great deal in common in their mechanism of action at the molecular level, the intimate details of the fit of substrate onto the sites of action differ. The 4-position in PAA seems to be more sensitive to substitution in a more complex way than POA. It would appear that the 4-substituents in each series find themselves in a different milieu at the receptor site than the 3-substituents. The fact that the PAA derivatives seem to consummate their 2-point attachment at the meta position rather than the ortho may mean that the substrate-receptor complex in the PAA series is shifted in a lipoprotein matrix so that electrostatic and steric effects of 4-substituents in the PAA series become more important. Åberg (1) found that larger substituents in the para position of phenoxyacetic acid progressively diminished the promotive

Table IV. *Relationship of Structure of 2- and 4-Substituted Phenylacetic Acids and Activity in Promoting Elongation*

Function	σ*	π*	Molar concn for +10% effect	Obsd $\log \frac{1}{C}$	Calcd** $\log \frac{1}{C}$
2-I	0.28	1.22	6×10^{-7}	6.2	6.54
2-Br	0.23	0.91	3×10^{-7}	6.5	6.34
2-Cl	0.23	0.68	7×10^{-7}	6.2	6.24
2-CH_3	—0.17	0.49	1×10^{-6}	6.0	5.25
2-CN	0.63	—0.28	5×10^{-6}	5.3	5.25
2-OCH_3	—0.27	0.04	5×10^{-6}	5.3	4.98
2-OH	—0.36	—0.54	1×10^{-5}	5.0	4.45
2-COOH				Inactive	
4-Br	0.23	0.91	1×10^{-5}	5.0	6.34
4-Cl	0.23	0.70	3×10^{-6}	5.5	6.26
4-CH_3	—0.17	0.45	1×10^{-5}	5.0	5.26
4-COOH				Inactive	
4-CN				"	
4-I				"	
4-NO_2				"	
4-$COCH_3$				"	
4-$NHCOCH_3$				"	

* See table I. Except for 2-OH function, π is that value for the function in the 3-position. Although π for the functions in the 2- and 3- positions of POA differs somewhat, this effect is much less in the PAA series.
** Calculated using equation VIII.

effect while such was not true for substituents in the meta position. Large groups in the para position will eliminate promotive effects on elongation regardless of values for σ or π (10). A similar effect exists for the phenylacetic acids, as shown in table IV, where the para position appears to be even more sensitive. The difference between the observed and calculated values for 4-BrPAA is 1.34 while that for 4-BrPOA (10) is only 0.64 and the difference for 4-ClPAA is 0.76 while that for 4-ClPOA is only 0.25. The fact that the 4-CH_3 derivative is not poorly predicted may indicate that the repulsive forces in the steric effect are highly electrostatic in nature since the methyl group is not much different in size from chloro. This is in line with the poor prediction for inhibitory action of the highly polar 4-nitro and 4-acetyl PAA functions.

In substituted benzoic acids the inactivating effect of large groups in the para position is even more pronounced. Although 2,5-dichlorobenzoic acid promotes elongation, 2,4-dichlorobenzoic acid is completely inactive (20). Since σ and π are only slightly different for chlorine in the 4- or 5-position, the inactivity of the 2,4-isomer must be due to a steric effect.

The evidence from the data for the phenoxyacetic acids, phenylacetic acids and benzoic acids indicates that the shorter the side chain holding the carboxyl group, the more susceptible the para position is to steric inhibition of the promotive effect on elongation.

The effect of the 4-substituent in the inhibitory action of the auxin may be in part determined by the length of the side chain holding the carboxyl group. For the phenoxyacetic acids the longer side chain places the 4-substituent in such a position that only hydrophobic bonding is possible. For the phenylacetic acids, with a shorter side chain, the 4-substituent is positioned so that electrostatic effects increase the degree of inhibitory action.

The inhibitory action of 2,4-dichlorophenoxyacetic acid has special interest in this connection. The sum of the π values for the 2 halogens in the molecule is 1.38 and substitution of this value in equation XII gives a calculated value of 3.99. The observed value for $\log \frac{1}{C_i}$ is 4.0 and thus the inhibitory effect of the molecule is qualitatively the same as for other POA derivatives.

It is of interest to compare the slopes and intercepts of equations XII and XVI. The values are extremely close indicating, as long as very highly polar substituents are absent in 4-PAA derivatives, precisely the same mechanism of action is involved. It is worth comparing these equations with those found for the inhibition of a variety of processes which are dependent on oxidative metabolism (13). Since π is defined as $\pi = \log P_x - \log P_H$ where P_x is the partition coefficient of a derivative and P_H that of a parent molecule, we can place equation XII on a logP basis since $\log P_H$ for POA = 1.21. Substituting $\pi = \log P_x - 1.21$ into equation XII yields equation XVIII.

$$\log \frac{1}{C_i} = 0.778 \log P_x + 1.971 \qquad \text{(XVIII)}$$

Equation XVIII can then be compared with equation XIX found (13) for the 50 % inhibition of cell division in *Arbacia* eggs by barbiturates.

	n	r	s	
$\log \frac{1}{C_i} = 0.801 \log P_x + 1.076$	19	0.960	0.171	(XIX)

The slopes indicating dependence on lipophilic character are strikingly close. The intercepts cannot be compared since in 1 case the effect is 50 % inhibition while in the other it is 5 % inhibition. For molecules which are isolipophilic, less is needed to bring about the result covered by equation XVIII than that covered by equation XIX. The similarity of equations XVIII and XIX and, in fact, the similarity of equation XVIII to many other equations (13) correlating inhibitory effects in biological systems suggests that supra-optimal concentrations of auxins inhibit electron transport in oxidative metabolism (13).

The 2-substituted phenylacetic acids were not included in the regression analysis of the promotion of elongation because of possible steric hindrance of the substituents with the side chain. It is well established that linear free-energy relationships often fail with ortho substituents (15) and, in the 2-substituted phenoxyacetic acids, the promotive effect on elongation was unpredictable. The data for the 2-substituted phenylacetics in table IV show, however, that 6 of the 7 compounds promoting elongation give just as good correlation as the meta and para substituted derivatives of table I on which the regression analysis was based. Thus it must be the interaction between the 2-substituents and the oxygen of the side chain of the POA derivatives which is not predicted by substituent constants, and in the PAA derivatives there is much less interaction of the 2-substituent and the inert $-CH_2-$ of the side chain. The lack of a steric effect by the 2-I function in PAA is impressive considering the large size of iodine.

Recent measurements of the infra-red stretch frequency in substituted indole-3-acetic acid molecules and its correlation with σ (22) support the long-standing hypothesis (7) that electron-withdrawing substituents activate the aromatic nucleus for auxin effects. When steric and lipophilic factors appear to be constant, promotive activity of the indole compounds parallels increased positive values of σ.

The electronic and lipophilic effects of single sub-

stituents in phenoxyacetic acids, phenylacetic acids and indoleacetic acids can be assessed with some degree of reliability. Now, using substituent constants, it will be possible to evaluate the steric effects in auxin molecules.

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