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AUXINS: ON THE NATURE OF THE RECEPTOR SITE AND MOLECULAR REQUIREMENTS FOR AUXIN ACTIVITY

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Key Word Index—Auxins; receptor site; structure–activity correlation.

Abstract—Suggested structural requirements for auxin activity are defined in terms of the receptor site with which the auxins interact. It is suggested that the site may be planar but for the portion which accepts the oxygen atoms of the carboxylic acid, and that compounds which have appropriate atoms covering postulated critical areas will have auxin activity. It is postulated that the area which accepts the indole nucleus is electrophilic in nature.

INTRODUCTION

Recently, a new approach to the problem of developing a more comprehensive structure–activity theory to account for the hormonal properties of the natural and synthetic auxins has been postulated [1]—the first for some years [2]. An endeavor to develop new correlations in this area is warranted, however, because it has been said that no comprehensive list of the structural and steric requirements necessary for auxin activity has been formulated which satisfactorily covers all molecules which are known to possess growth promoting properties [2]. This is despite the fact that synthetic auxins have been known for some decades [3]. The development of the concept of antiauxins [4] has also been inadequate [5]. The importance of the development of a comprehensive theory is further indicated by the recent work of Hertel, Batt, Venis and their coworkers [6–9], who have demonstrated the presence of at least two different specific auxin receptors, and conclusions as to what may be the chemical nature of such receptors may assist in their isolation and characterization. The synthesis of new auxin agonists and antagonists would also be assisted, as would the rational design of new agricultural chemicals and plant growth regulators.

The theories so far put forward endeavor to explain auxin activity in terms of three properties of the molecules under consideration: (1) the chemical capacity to form covalent bonds with the receptor site; (2) the electronic properties which affect their ability to bind to the receptor site, whether the binding be covalent or non-covalent in nature: and (3) stereochemical properties. The inadequacies of the earlier theories are well known and have been adequately discussed elsewhere [2, 5, 10]; the main criticisms may be summarized as follows.

The two point attachment theory of Hansch and Muir [11] is probably not correct, because it involves bond formation between the active site and an aromatic ring, and in many cases this requires the displacement of substituents which differ widely in their electronic properties, and thus their ability to be displaced. Further, recent work on auxin receptor sites would indicate that the binding of the IAA molecule may be non-covalent in nature [6–9]. The charge separation theory of Thimann and Leopold [12]* postulates that in order to fit into an attachment site, the auxin molecule needs a negative charge and a positive charge at a specific distance (5.5 Å) from one another. The negative charge is considered to be that of the ionized carboxyl group and the positive charge being either on an unsubstituted position of an aromatic ring or another appropriate atom. While this can account for most substitution patterns, and could apply whether the binding is covalent or non-covalent, there are some exceptions. For example, Porter and Thimann [13] have pointed out that the low activity of 5,7-dichloro IAA,† **1-15** is a serious deviation from the theory. Also in some cases activity is retained when the required unsubstituted position on the aromatic ring is in fact substituted. Stereochemical considerations are also beyond the scope of this theory.

The three point attachment theory of Smith and Wain [14] does not explain the activity of the arylbenzoic acids nor the differing substitution patterns on the aromatic rings of the various synthetic auxins. While a stereochemical explanation is offered for the *R* and *S*‡ forms of the aryl α-propionic acids, the exception of the *R* and *S* 3-α-indolylpropionic acids **8-1** and **8-2**, where both forms are active, together with other exceptions, cannot be accounted for. The conformational change theory of Kaethner [1] is also stereochemical in nature. A three-dimensional receptor site is postulated, which is of such a size and shape as to prevent many compounds which would otherwise be expected to be active, from interacting. A simultaneous complex conformational change by both the receptor site and the interacting molecule are postulated. The proposed recognition conformation **1-1a** and the modulation conformation **1-1b** of the IAA molecule are shown in Fig. 1. To be active, all molecules must be able to undergo an analogous conformational change, while those which, for con-

* Note added in proof: A modification of the charge separation theory has now appeared [57].

† Abbreviations: IAA: indoleacetic acid; NAA, naphthylacetic acid, 2,4-D: 2,4-dichlorophenoxyacetic acid.

‡ The Cahn Prelog Ingold system of stereochemical nomenclature is used [15].

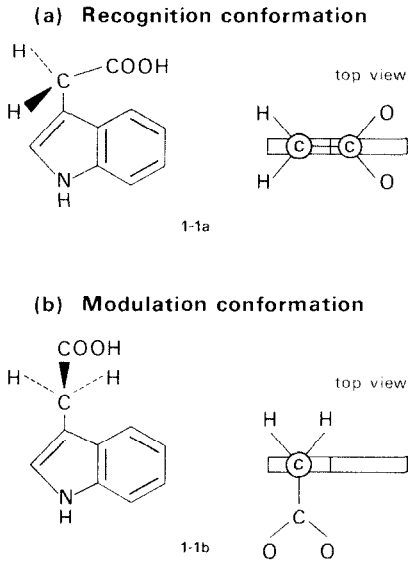


Fig. 1. Recognition and modulation conformations of the IAA molecule as required by the conformational change theory.

figurational or conformational reasons cannot undergo such a change, are inactive. Even this theory has some deficiencies, however. Firstly, as the author himself points out, the structural requirements of the benzoic acids cannot yet be fully explained by the theory from the presently available data. In addition, it is apparent that other than steric factors must be having significant effects. By way of example, if conformation **1-1a** is the recognition conformation for IAA molecules, it might be expected, on steric grounds alone, that 4-chloro IAA **1-3** would be less active because the electron rich chlorine atom would be adjacent to the negatively charged oxygens of the carboxyl group, yet it is in fact more active than the parent compound (see Table 1). Similarly, steric interference is proposed as the reason for the low activity of 2-methyl IAA **1-13** because the methyl group interferes with the conformational change of that molecule. On this ground, 2-chloro IAA **1-2** might also be less active, yet again it is more active than IAA itself and almost thirty-fold more active than 2-methyl IAA. While conformational change can account for most activities of the asymmetric arylpropionic acids, as with the three point attachment theory, the exceptional activities of the enantiomeric indolyl, thianaphthene- and naphthyl-propionic acids, as well as that of indole*iso*butyric acid, still cannot be accounted for. As has been previously pointed out, these exceptions are of significance [10] and on this ground alone, further investigation is warranted [10]. At least some of these inadequacies do not appear to be amenable to resolution by the approaches so far taken.

Recently a structure–activity correlation was postulated for compounds which affect the geotropic response in plants, which also appears to apply to auxin transport inhibitors [16, 17]. It was found that substituents on an aromatic ring had two important effects. Firstly, if the effect of substitution was to increase the size of the active molecule up to a certain minimum size, then activity was increased, whether the substituent was electron withdrawing or donating in character. Secondly, fused aromatic rings, aromatic rings, and chlorine were particularly effective in increasing activity. To account for these observations it was postulated that binding to the active site in the area covered by the aromatic rings or substituents could be by means of a π bonded

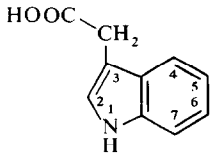
Table 1. Auxin activity of indoleacetic acid derivatives

Compound No.	Compound	Coleoptile cylinder	Relative* activity	Pea test	Relative† activity
1-1	3-Indoleacetic acid	+++‡	100	+++§‖	100
1-2	2-Chloro IAA				350
1-3	4-Chloro IAA	+++‡	140	+++§	700
1-4	5-Chloro IAA			+++§	266
1-5	6-Chloro IAA			+++§	500
1-6	7-Chloro IAA			++§	200
1-7	5-Fluoro IAA			+++§	120
1-8	7-Aza IAA			+++†	100
1-9	5-Methoxy IAA			++¶	
1-10	6-Methoxy IAA			++¶	
1-11	7-Methoxy IAA			++¶	
1-12	1-Methyl IAA	+**		+§	13
1-13	2-Methyl IAA	+	1.5	+§	12
1-14	5-Methyl IAA	++**		++§	60
1-15	5,7-Dichloro IAA		1.5	++§	15
1-16	5,7-Dichloro-2-methyl IAA	+‡			
1-17	1-Indolecacetic acid				(root auxin)††
1-18	4-Indoleacetic acid	+‡‡			

* Ref. [42] Relative activity $= \frac{\text{Molar concn of IAA inducing 0.15 mm elongation}}{\text{Molar concn of compound inducing 0.15 mm elongation}} \times 100.$

† Ref. [13] Relative activity $= \frac{\text{Concn of IAA required to reduce outward curvature by } 100^\circ}{\text{Concn of compound required to reduce outward curvature by } 100^\circ} \times 100.$

‡ Ref. [42]; § Ref. [45]; ‖ Ref. [46]; ¶ Ref. [47]; ** Ref. [43]; †† Ref. [31]; ‡‡ Ref. [44].



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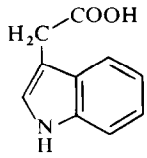


1-1a

interaction or charge transfer complex. In this explanation it is implicit that the chlorine atom itself could bind to the active site by virtue of the fact that it is an electron rich atom and therefore able to mimic to some extent the binding capabilities of the π electrons of aromatic rings. These two factors, viz. increased coverage of the active site and possible binding of the substituents to the site, do not appear to have been previously considered in relation to the structure–activity of auxins. Because an aromatic ring is at least highly desirable for auxin activity, and halogens in general are known to increase activity, these factors have been incorporated into an alternative and more comprehensive structure–activity correlation which is discussed below.

Activity tables

A vast number of compounds are now known to have auxin activity, and it was found unnecessary for present purposes to include them all, particularly as comprehensive tables have already been collated by Jonsson [18]. In the tables presented here, 0 = no activity, while the range +, + +, and + + + represents measureable (slight) activity, low activity, and activity within an order of magnitude of the activity of IAA, respectively. This method of assessment is generally consistent with Jonsson's tables so that direct comparisons can be made. Results which were obtained by using coleoptile straight growth or split pea stem assays have been used where possible, since these are the generally accepted methods for assessment of auxin activity [10]. Many of the other methods of assessment, particularly the root growth assays, are not specific for auxins, and can be misleading [10]. Assessment of relative activities is rendered difficult by many factors, including variations between different bioassays, and the differing methods of presenting results used by different workers. Where possible and appropriate, therefore, results of individual workers have been included where they have assayed large numbers of compounds.

DISCUSSION

1. *The auxin receptor site*

Since the auxin receptor site is *ex hypothesi* complementary to the IAA molecule, it can be represented as in Fig. 2. It will be argued that the area which accepts the indole ring is electrophilic in nature, and extends beyond the boundaries defined by the indole ring. This whole area is therefore divided into areas designated Ar_1 and Ar_2, corresponding to the pyrrole and benzene portions of the molecule respectively, while the surrounding areas are marked a–f. The area corresponding to the methylene carbon of the IAA molecule is designated the α area, with the remaining essential part being referred to as the carboxyl acceptor.

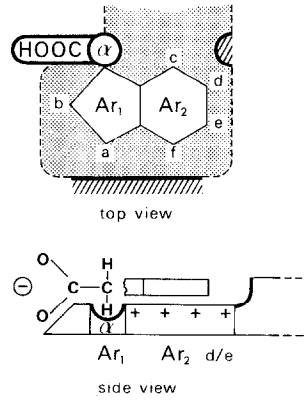


Fig. 2. The auxin receptor site.

The electron acceptor

It is suggested that, if the requirement for the carboxyl acceptor and α area are met, then subject to any size, shape and conformational requirements imposed by the receptor site, auxin activity will result when the areas Ar_1 and Ar_2 are covered by the interacting molecules. The degree of activity will depend on the ability to bind to the acceptor. This in turn will depend upon size and electron availability of the interacting molecule. It is suggested that the area which binds to the indole ring is electrophilic in nature and can interact with the π electrons of aromatic rings and other electron rich atoms. The binding may be non-covalent in nature. The fact that monosubstitution by chlorine on each of the carbon atoms of 3-IAA (**1–1**) causes an increase in auxin activity in all cases [13] (Table 1) would indicate that substituent binding is at least possible, and can outweigh substituent effects on the electron distribution in aromatic rings. In approximate decreasing order of effectiveness, some atoms and groups which can give rise to auxin activity are: fused aromatic rings, aromatic rings Cl, F, Br, I, CH_3, which is not inconsistent with the above interpretation of electron availability. Examples of fused ring compounds are 3-IAA itself and 1-NAA (Tables 1 and 2). The effect of substituents on molecules is to tend to increase activity if they have available electrons to bind, but to decrease activity if they have not. Examples of this effect are shown in **1-1** to **1-14**, Table 1, **2-18** and **-19** (Table 2) and in phenoxy acids, Table 5. It should be noted that only aromatic rings and halogens give rise to high activity; other substituents have only comparatively weak effects.

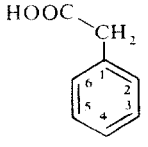
While this postulate is at variance with the charge separation theory it would seem *a priori* more likely that a site which can bind with a large number of differing aromatic rings would be electrophilic rather than nucleophilic in nature, being able to interact with the delocalized π electrons of the rings. Charge distribution may not be unimportant, however. For example, while 3-IAA and 1-NAA have similar activities, it should be noted that 1-IAA (**1-17**) and 4-IAA, (**1-18**) are significantly less active. The generally low activity of NO_2 substituted compounds may also be due to the powerful electron withdrawing properties of the group being able to reduce the electron density of the aromatic rings, thus diminishing their ability to bind to the active site.

Table 2. Auxin activity of arylacetic acids

Compound No.	Compound	Coleoptile cylinder	Relative activity*	Pea test
2-1	Phenylacetic acid	+†	1	+†
2-2	2-Nitro PAA			0‡
2-3	3-Fluoro PAA	+†	1.5	
2-4	3-Nitro PAA			1‡
2-5	4-Amino PAA	+§	0.05	
2-6	4-Fluoro PAA	+§	1.5	
2-7	4-Iodo PAA	0§	0	
2-8	4-Nitro PAA	0§	0	
2-9	4-Phenyl PAA	0§	0	
2-10	2,3-Dichloro PAA	+++‖		+++‖
2-11	2,4-Dichloro PAA			+¶
2-12	2,6-Dichloro PAA	++‖		+++‖
2-13	2,4-Dimethyl PAA	+§	0·5	
2-14	3,5-Dimethyl PAA	+§	0·5	
2-15	2,4-Dinitro PAA	0§	0	
2-16	2,3,6-Trichloro PAA	++‖		+++‖
2-17	2,4,6-Trimethyl PAA	0§	0	
2-18	1-Naphthaleneacetic acid	+++†§	50	+++†
2-19	2-Chloro NAA			+++**
2-20	8-Chloro NAA	0††		0**††
2-21	2-Naphthaleneacetic acid	+†		+†
2-22	2-Phenanthreneacetic acid	0§	0	
2-23	3-Phenanthreneacetic acid	0§	0	
2-24	9-Phenanthreneacetic acid			+‡‡

* Ref. [42] defined in Table 1; † Ref. [48]; ‡ Ref. [3]; § Ref. [42]; ‖ Ref. [49]; ¶ Ref. [55];** Ref. [31]; †† Ref. [51]; ‡‡ Ref. [50].

Coverage of at least part of the electron acceptor is postulated as being required for auxin activity, and selected compounds which illustrate this concept are shown in Diagram 1. If Ar_1 only is covered, such compounds exhibit only weak activity or no activity. This can be seen in phenylacetic acid **2-1**, 2-naphthylacetic acid **2-21** (Diag. 1), the *ortho*-substituted benzoic acids,



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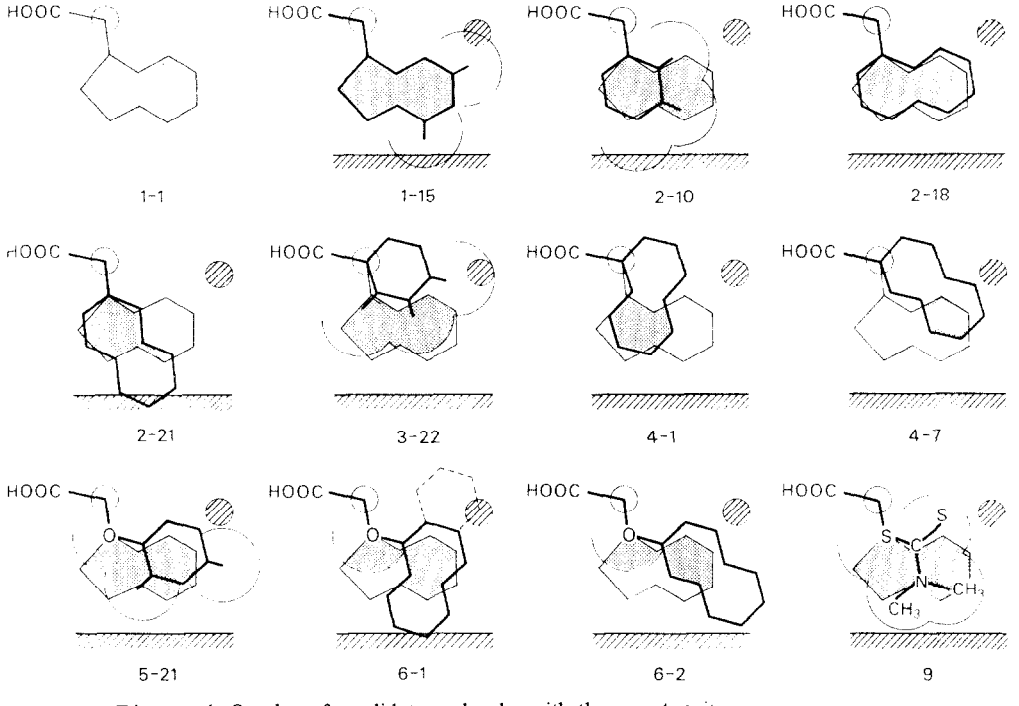
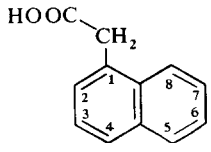


Diagram 1. Overlap of candidate molecules with the receptor site.

Table 3. Auxin activity of arylcarboxylic acids

Compound No.	Benzoic acid derivative	Coleoptile cylinder*	Relative activity†	Pea test
3-1	Parent compound	0	0	
3-2	2-Fluoro	0	0	
3-3	2-Chloro	+	0.05	
3-4	2-Bromo	+	0.1	
3-5	2-Nitro	+	0.1	
3-6	2-Amino	0	0	
3-7	2-Iodo	0	0	
3-8	2-Methyl	0	0	
3-9	2,3-Dichloro	++‡		+‡
3-10	2,4-Dichloro	0	0	0§
3-11	2,5-Dichloro	+*	1.0	
3-12	2,6-Dichloro	+‡	0.1	0§
3-13	2-Chloro-5-bromo	++‖		++‖
3-14	2-Chloro-5-iodo	+++‖		++‖
3-15	2-Bromo-5-chloro	++‖		++‖
3-16	2,5-Dibromo	++‖		++‖
3-17	2-Bromo-5-iodo	+++‖		+‖
3-18	2-Iodo-5-chloro	+‖		0‖
3-19	2-Iodo-5-bromo	+‖		0‖
3-20	2,5-Diiodo	+‖		0‖
3-21	3-Chloro-2-Iodo	++¶		+¶
3-22	2,3,4-Trichloro	0‡		0§
3-23	2,3,5-Trichloro			+§
3-24	2,3,6-Trichloro	+++§		+++‡
3-25	2,4,5-Trichloro			0§
3-26	2,4,6-Trichloro			0§
3-27	3,4,5-Trichloro			0§
3-28	2,3,5-Triiodo	+++*?	50	
3-29	2,3,6-Trichloro-4-fluoro	++¶		++¶
3-30	2,6-Dichloro-4-fluoro-3-nitro	++¶		++¶
3-31	2-Bromo-3-nitro			Active**
3-32	3-Amino-2,5-dichloro	++††		
3-33	2,5-Dichloro-5-methoxy	+++††		
3-34	2,5-Dichloro-3-nitro	+††		

* Unmarked, ref. [42]; † Ref. [42] defined in Table 1; ‡ Ref. [49]; § Ref. [24]; ‖ Ref. [28]; ¶ Ref. [25]; ** Ref. [3]; †† Ref. [56].



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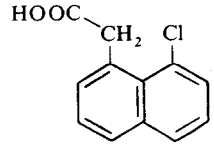

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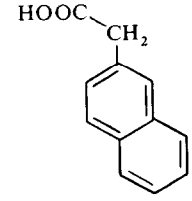

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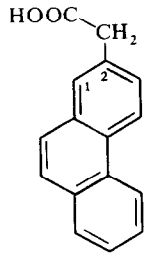

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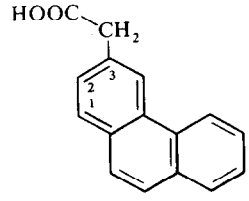

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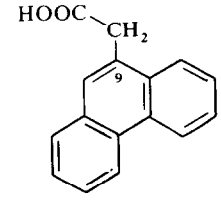

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Table 4. Auxin activity of fused ring arylcarboxylic acids

Compound no.	Compound	Coleoptile* cylinder	Pea* test
4-1	1-Naphthoic acid		+†
4-2	2-Chloronaphthoic acid		+ +†
4-3	8-Chloronaphthoic acid	+ +	+ +
4-4	8-Bromonaphthoic acid		+ +
4-5	8-Iodonaphthoic acid		+ +
4-6	8-Methylnaphthoic acid		+ +
4-7	2-Naphthoic acid		0†
4-8	9-Anthracenecarboxylic acid		0‡

* Unmarked, ref. [25]. † Ref. [39]. ‡ Ref. [52].

3-2 to **3-8** (Table 3) and 1-naphthoic acid, **4-1** (Table 4 and Diag. 1). If only Ar_2 is covered, activity is also weak, as in phenoxyacetic acid **5-1**, (Table 5) and the naphthoxyacetic acids **6-1** and **2** (Table 6 and Diag. 1). The 2,5-disubstituted benzoic acids could either cover Ar_1 or Ar_2 depending on which way the molecule fits to the site. Except for the 2-iodo derivatives, (see below) their activity can be accounted for by either analysis. The way in which benzoic acid molecules may cover the active site is also illustrated by **3-22** in Diagram 1.

Where Ar_1 only is covered by an aromatic ring, substitution which has the effect of covering Ar_2 generally increases activity. In the arylacetic acid series, monosubstitution in the 2 or 3 positions only gives weak activity, but the 2,3-disubstituted derivative **2-10** has high activity, so too does 1-NAA, which can also be regarded as a 2,3-disubstituted phenylacetic acid (see Table 2 and Diag. 1). In the *ortho*-substituted arylbenzoic acids, activity is also increased by the addition of a further substituent in the 3-position as in **3-3** and **3-9**, **-21**, **-23**, **-24**, **-28**, **-29**, **-31**. The auxin activities of the herbicides amiben **3-32**, dicamba **3-33**, and dinoben **3-34** are also not inconsistent with this hypothesis. On the basis of increased coverage, it would be predicted that 5-chloro-1-naphthoic acid would have

Table 5. Auxin activity of phenoxyacetic acids

Compound no.	Phenoxyacetic	Coleoptile cylinder*	Relative †	Activity ‡	Pea test§
5-1	Parent compound	0‖	0.03	100	0
5-2	2-Chloro	+‖	0.06	140	+
5-3	3-Chloro	+ +‖	2.0	130	+ +
5-4	4-Chloro	+ +‖	5.0	145	+ +
5-5	2-Bromo	+	0.1	145	
5-6	3-Bromo	+ +‖	2.5		
5-7	4-Bromo	+	1.5		
5-8	2-Iodo	+	0.1		
5-9	4-Iodo	0	0		
5-10	2-Methoxy	0	0		
5-11	3-Methoxy	+	0.1		
5-12	4-Methoxy	+	0.03		
5-13	2-Methyl	+	0.2		
5-14	3-Methyl	+	0.07		
5-15	4-Methyl	+	0.05		
5-16	2-Nitro	0	0		
5-17	3-Nitro	+	0.2		
5-18	4-Nitro	+	0.1		
5-19	2,4-Dibromo	+ +	12.5		
5-20	2,3-Dichloro	+‖		115	+ +
5-21	2,4-Dichloro	+ + +‖	2.5	155	+ + +
5-22	2,5-Dichloro	+ +‖		140	+ +
5-23	2,6-Dichloro	0	0	80	+
5-24	3,4-Dichloro	+ +		140	+ + +
5-25	3,5-Dichloro	0	0	80	0
5-26	2,3,4-Trichloro			130	+ + +
5-27	2,3,5-Trichloro			110	+
5-28	2,3,6-Trichloro	+¶		90	+ +¶
5-29	2,4,5-Trichloro	+ + +‖	25	45	+ + +
5-30	2,4,6-Trichloro	0‖	0	90	0
5-31	3,4,5-Trichloro	+**		105	+
5-32	2,4-Dichloro-5-nitro	+	0.2		
5-33	2,4-Dichloro-6-fluoro	+ + +**			+ + +**
5-34	2,4-Dichloro-6-bromo	0**			0**
5-35	2,4-Dimethyl	+**	0.5		+ +**
5-36	2,5-Dimethyl	+	0.2		
5-37	2,6-Dimethyl	0**			0**
5-38	3,5-Dimethyl	0**	0		0**

* Unmarked, ref. [42]; † Ref. [42] defined in Table 1.
‡ Calculated from ref. [53]

$$= \frac{\text{Extension due to compound}}{\text{Control extension}} \times 100 \text{ measured at 5 ppm.}$$

§ Unmarked ref. [33]; ‖ Ref. [33]; ¶ Ref. [49]; ** Ref. [34].

Table 6. Auxin activity of naphthoxyacetic acids

Compound No.	Compound	Coleoptile cylinder	Pea test	Tomato
6-1	1-Naphthoxyacetic acid	0*	+*	
6-2	2-Naphthoxyacetic acid	++*	++*	++†
6-3	1-Chloronaphthoxyacetic acid			+*
6-4	3-Chloronapthoxyacetic acid			+++†

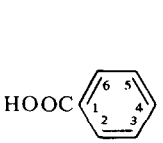
* Ref. [33]. † Ref. [54].

enhanced activity. Where Ar_2 only is covered, substituents covering Ar_1 will also increase activity. In the phenoxyacetic acid series, *ortho* substitution by Cl, Br, I, CH_3 but not NO_2 increase activity (Table 5): compare also **6-2** and **6-4**. The activity of the substituted aryl carboxylic acids shows that it is not necessary for an aromatic ring to overlap Ar_1 or Ar_2, (see Diag. 1) and the activity of the dithiocarbamates and their analogues **9**, **10**, **11**, [19–22] would indicate that an aromatic ring need not be present at all. If Ar_1 and Ar_2 are already covered, the general effect of introducing substituents which can bind to the acceptor is to increase activity. This can be seen in both the substituted indoleacetic acids (Table 1) and in the phenoxyacetic acids (Table 5).

Since all aromatic rings and aromatic rings with single atom substitutents which form part of active molecules must of necessity be planar, the conformation of the electron acceptor must also either be planar or capable of interacting with planar groups. It can be noted that the most stable conformations of the active dithiocarbamates and related compounds would be expected to be an open chain form with the dithiocarbamate portion planar and conjugated. They would then be able to lie over Ar_1 and Ar_2. The dithiocarbamate **9** is also illustrated in Diagram 1.

Size and shape of the electron acceptor

The increased activity of all the mono-chlorinated indoleacetic acids over the parent molecule would suggest that the electron acceptor extends beyond the boundary defined by the indole ring itself. However, while, it is large enough and non-specific enough to accommodate a wide range of synthetic auxins, there are size and shape limitations which can prevent otherwise active molecules from interacting. It is suggested that steric obstruction occurs in an area between the c and d areas, and also in the a and f areas. This steric effect can be observed in the four major classes of synthetic auxins (Tables 1–6). The effect is manifested by reduced activity when a molecule impinges on the obstructed areas. Veldstra has suggested that the reason for the inactivity of the *para* substituted benzoic acids is steric in nature [23, 24, 25]. 4-Chloro derivatives, despite the generally activating effect of the chlorine, are inactive, yet the smaller 4-fluoro derivatives with similar electronic properties retain activity. Compare **3-9** and **3-10**, **3-3** and **3-22**, **3-12** and **3-26**, **3-24** and **3-29**. The 4-fluoro derivative **3-30** is also active. Consistent with this hypothesis, chlorine substitution which would impinge on this area in the IAA series and the phenoxyacetic acid series either does not increase activity, or increases it less than chlorine substitution in other areas. It would appear that the site can still accommodate such molecules because activity is not lost completely. However, if there is disubstitution with the second substituent appearing in area f, the electron donor is too large to fit on the acceptor and activity is considerably reduced. This is again illustrated in Diag. 1. The effects are exemplified in **1-4**, **1-6** and **1-15** of the IAA series, while in the phenoxy series **5-2** can be compared with **5-20**, **5-21** and **5-27**; **5-22** with **5-25**, and both **5-29** and **5-21** with **5-26**. The 3,5-dichloro derivative **5-25** is inactive. In comparison, *meta* substitution tends to increase activity rather than reduce it in both the arylacetic acid and arylcarboxylic acid series. A steric limitation in the a area is also indicated by the inactivity of arylacetic acids substituted in the 4-position with large groups, while sterically smaller groups substituted in the same position do not destroy activity. Similarly, 1-methyl-3-IAA (**1-12**) retains some activity, while 1-acetyl-3-IAA is inactive [5]. The complete inactivity of the 2- and 3- phenanthreneacetic acids **2-22** and **-23** can also be explained by postulating steric obstruction in the a and f areas. The low activity of the 9-phenanthrene acid **2-24** would not be inconsistent with this hypothesis: but for steric hindrance in the a area, this latter molecule would be expected to be as active as 1-NAA, because it covers both Ar_1 and Ar_2 areas. It can be seen that the differences in activity between 1- and 2-naphthylacetic, naphthoic, and naphthoxyacetic acids, which have hitherto been difficult to explain, can be accounted for by the coverage of area and size and shape limitations postulated above. It is suggested that the more active member of each pair can be predicted by comparison of structures **2-18** and **2-21**; **4-1** and **4-7**; **6-1** and **6-2**; as they are shown



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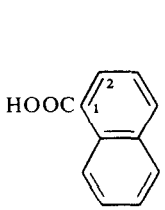

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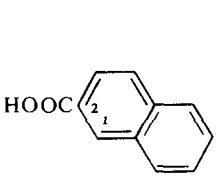

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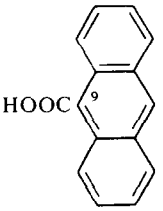

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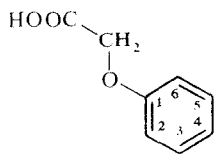

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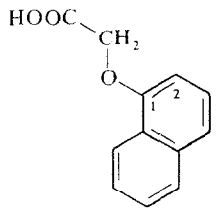

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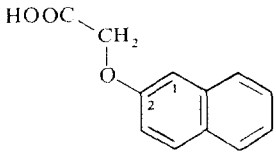


6-2

in Diagram 1. The herbicide picloram **12** is an apparent anomaly because it has been found to have auxin activity [26] yet it has a 3-chloro substituent which would be expected to impinge on a sterically hindered area. Whether this is a true exception would depend on the activity of the analogous molecule unsubstituted in this position, which would be predicted to be more active.

Lack of steric obstruction in the d and e areas is indicated by the activity of 2-naphthoxyacetic acid **6-2**. In 2,4-D, (**5-21**), the 4-chloro substituent is placed in the d–e area, and activity is enhanced. The activity of the arylcarboxylic acids would tend to indicate lack of steric obstruction in the c area, at least for some distance. However the lower activity of the 9-anthracenecarboxylic acid **4-8** as compared with 1-naphthoic acid **4-1** may indicate that the limit has been reached in this case. The activity of the 8-substituted naphthoic acids **4-3**, **-4**, **-5**, **-6** would indicate that the steric limit of the b area has not yet been reached.

The carboxyl acceptor

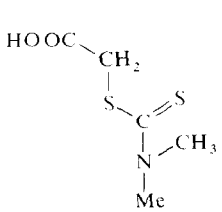
That a carboxylic acid or other acidic group which can mimic it, is essential for activity is well known [10]. It has been pointed out by Veldstra [23, 24, 27] that the enhanced activity of the 2,6-disubstituted arylcarboxylic acids (**3-12**, **-24**, **-29**, **-30**) may be due to the steric effect of the substituents in forcing the carboxyl oxygens out of plane with the aryl ring, which would be their normal position because of conjugation. Consistently, the 2- and 8-substituted naphthoic acids **4-2** to **-6** show increased activity. The dipole of the carboxyl group would thus be at an angle to the remainder of the ring. It should be noted that replacement of 2-chloro- and 2-bromo substituents by 2-iodo markedly reduces activity in the 2,5-disubstituted arylcarboxylic acids. This can be seen in compounds **3-13** to **3-20**, and similar effects have been observed in the 2,3,5- and 2,3,6-substituted benzoic acids [28]. This has been interpreted as being due to a steric inhibition of the attachment of the carboxyl group to its receptor unit (i.e. carboxyl acceptor) [28]. The conformational requirements of the receptor site may therefore be highly specific in this area, and would determine how the remainder of the molecule could interact with the rest of the site.

The α area

The atom impinging on this area can be either tetravalent carbon, e.g. a methylene group as in IAA, the arylacetic acids and phenoxyacetic acids: trigonal carbon, as in the arylcarboxylic acids, atropic acids, **14** R=H, **14** R=CH_3, [22] and *trans*-tetrahydronaphthylidineacetic acid **15**, [23]. The thio analogue of IAA, (**16**), however, is inactive [29]. The herbicidal properties of the phenoxy acrylate **17** [30] may well be due to its possession of auxin activity. The α atom here is again trigonal carbon. It has been suggested [31] that it is not the presence of a methylene hydrogen which gives rise to activity in this area but rather the absence of steric interference. Consistent with this, the α-difluoro derivative of 2,4-D, (**18**) possesses less activity than its unsubstituted analogue [32] while 2,4-dichlorophenoxyisobutyric acid **20** only exhibits weak activity [33]. The α area may therefore exert a steric effect, and it is possible that it does not bind to the α atoms.

Conformation and relative configuration

The fact that the arylcarboxylic acids can be highly active would appear to preclude any configuration of the receptor site in which the carboxyl acceptor was in the c area, that is, it could accept an IAA molecule in conformation **1-1a**, Fig. 1. Further, because in the arylcarboxylic acids all carbon atoms and single atom substituents are of necessity coplanar, the receptor site must be able to interact with molecules having the carboxyl carbon, α atom (the atom overlying the α-area), and aromatic ring coplanar. The receptor site could therefore be as is represented in Fig. 2. The seven-fold increased activity of 4-chloro IAA **1-3** over the parent molecule would also support this conclusion. In conformation **1-1a**, the negatively charged oxygens of the carboxyl group would be adjacent to the electron rich chlorine atom, and there would be expected to be steric resistance resulting in reduced, not increased, activity.



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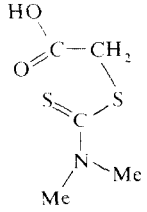

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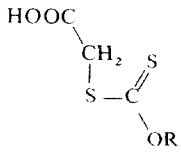

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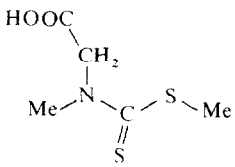

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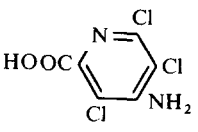

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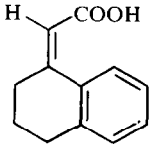

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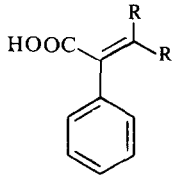

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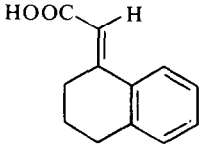

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It has previously been suggested that substituents in the 6-position of the phenoxy acids **5-23**, **-28**, **-30**, **-37** and in the 8-position of the 1-naphthylacetic acids **2-20** reduce activity because of their steric effects [33, 34]. The nature of these steric effects may therefore be to prevent the carboxyl carbon from becoming coplanar or adopting an appropriate conformation with respect to the rest of the molecule by interaction with the hydrogen atoms on the α-atom. This is shown in Fig. 4. A 4-chloro-substituent on the indole molecule **1-3** does not appear to have this effect, but models would show that the interatomic distances between the halogen and the methylene hydrogens is much greater in the indole molecule than in the phenoxy acids **5-23** and napthylacetic acids **2-20** (Table 7). It can also be seen that 6-fluoro substitution in the phenoxy acid **5-33** does not destroy activity, presumably because it is sufficiently small not to interfere. The shape of the receptor site may therefore be planar but for that part which accepts the oxygen or -OH atoms of the carboxyl group.

Absolute configuration

If the configuration of the receptor site is as shown in Fig. 2, two enantiomeric forms are possible, one as represented in Fig. 2, and the other its mirror image. Symmetrical auxin molecules could fit either. The well established rule for optically active phenoxy and aryl α-propionic acids is that the forms with the same

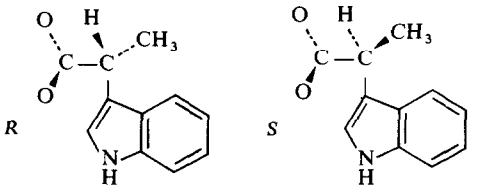
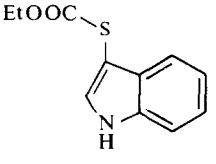


Fig. 3. Enantiomers of indole-α-propionic acid.

absolute configuration as the *S* (D) forms of indolepropionic acid **8-1**, Fig. 3 are far more active than their mirror images [35–37]. The active enantiomers are thus the *S*-arylpropionic acids and the *R*-phenoxypropionic acids.* On the model proposed here, they would fit the active site as in Fig. 2 rather than its enantiomer, because they would present only a hydrogen to the α area rather than the bulky methyl group. The lower activity of the enantiomeric forms would indicate that at least the great majority of the active sites have an absolute configuration complementary to the *S* forms of the arylpropionic acids. There are, however, important exceptions to the rule on which this conclusion is based. The main exceptions are the enantiomers of indole-α-propionic acid, **8–1** and **8–2**, and 1-naphthalene-α-propionic acid, **8–3** and **8–4** (Table 8), where the *R*-enantiomers have activities at least comparable to the activities of the *S*-enantiomers.

It is suggested that the explanation of these exceptions is also a steric one. Three factors are involved: the steric obstruction by the α area, the strength of the binding to the electron acceptor, and the conformational stability of the interacting molecule. As concluded above, there is steric interference between a methyl group and the α area. For the unfavourable enantiomers to bind to the site, therefore, the methyl group must be moved away from the α area, and in doing so it tends towards a conformation where it is coplanar with the aromatic ring. This would be a strained conformation, and it is suggested that the amount of resistance to attaining this conformation is a factor which determines their



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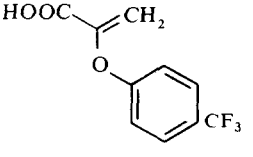

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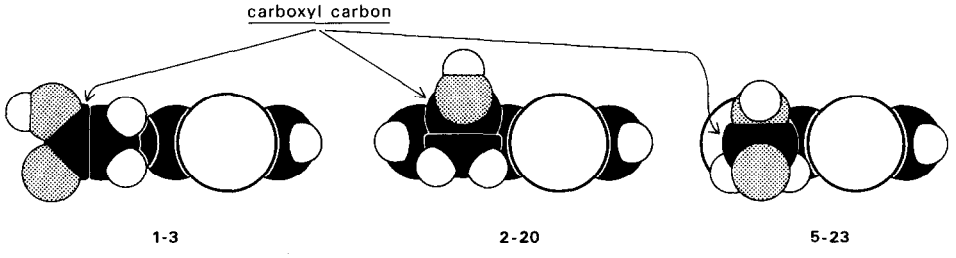


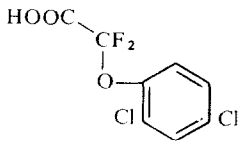
Fig. 4. Conformations of sterically hindered molecules.

* The rules of stereochemical nomenclature [15] require that the active enantiomers of the arylphenoxy acids be designated as *R*, although they have the same absolute configurations as the *S*-arylpropionic acids.

Table 7. Interatomic distances between chloro atom in c area and α carbon and correlation with auxin activity

Compound No.	Compound	Distance* Å	Coleoptile cylinder	Pea test
1-3	4-Chloro-3-indoleacetic acid	3.2	+ + +	+ + +
2-20	8-Chloro-1-naphthylacetic acid	2.3	0	0
5-23	2,6-Dichlorophenoxyacetic acid	2.1	0	+

* As measured on Dreiding models.



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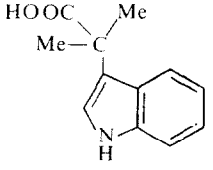

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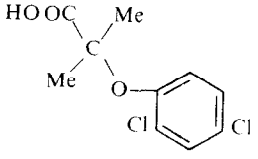

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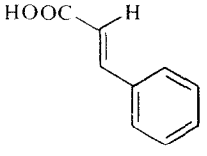

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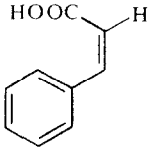

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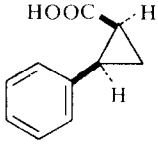

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activity. The higher the resistance the lower the auxin activity will tend to be, and the greater the difference between the activities of the enantiomers. A guide to the degree of the resistance can be obtained by measurement of the distance between the atomic centres of the carbon of the methyl group and the hydrogen atom in the c area in the strained conformation of the molecule. The shorter the distance, the greater the steric strain would be expected to be. Approximate distances are shown in Table 8, together with auxin activities and calculated activity ratios. It can be seen that they are not inconsistent with the above hypothesis. The activities of the enantiomeric pair of α-3 thianaptheneproprionic acid are also exceptional [37], but, also consistently, the interatomic distance measurement would be expected to lie in between those of the naphthyl- and indolepropionic acids. The α-phenylpropionic acids (**8-9** and **8-10**) would also be expected to have a low resistance to conformational change and thus a low activity ratio. Here the binding to the electron acceptor would also be expected to be weak (Ar_1 only is covered) and perhaps insufficient to overcome the resistance to the change. Results for these compounds are also shown in Table 8. The activity of indole*iso*butyric acid **19** and the comparative inactivity of the phenoxy*iso*-butyric acid **20** together with other phenoxy*iso*butyric acids in the wheat cylinder test [33] can similarly be explained by steric considerations, although the activity shown by 2,4,5-trichlorophenoxy*iso*butyric acid is perhaps anomalous.

Exceptions

With respect to the cinnamic acids, it has been shown that the natural (*trans*) isomer **21** may be an auxin antagonist [38], while the *cis* isomer **22** has auxin activity [23]. While it is possible that the *trans* isomer could be an antagonist, on the theory proposed here, the activity of the *cis* isomer is exceptional, unless partial coverage of the Ar_1 area, together with coverage of the b area, is sufficient to bestow activity. It is suggested,

Table 8. Interatomic distances between β carbon of α-propionic acids and hydrogen in c area in the strained (eclipsed) conformations and correlation of auxin activity between enantiomeric pairs

No.	Compound	Distance Å*	Activity Coleoptile cylinder†	Flax root†	Activity ratio: *R*/*S*
8-1	*S*(D)-α-3-indole propionic acid	2.3	+ + +	0.02	0.5
8-2	*R*(L) isomer		+ + +	0.01	
8-3	*S*(D)-α-1-naphthalene propionic acid	1.6	+ + +	0.15	0.67
8-4	*R*(L) isomer		+ +	0.10	
8-5	*R*(D)-α-2,4-dichlorophenoxy propionic acid	0.8	+ + +‡	0.033§	2.5×10^{-3}
8-6	*S*(L) isomer		0‡	13§	
8-7	*S*(D)-α-phenylpropionic acid	2.2‖	+ +	3	23
8-8	*R*(L) isomer		inhibitor	70	

* Measured on Dreiding models. † Unmarked ref. [37]. ‡Ref. [36]. § Ref. [35]. ‖Distance between β-carbon and 2-hydrogen.

however, that this isomer conforms to some of the rules which define an auxin transport inhibitor [17], and its effect may be due to its activity in this regard. A similar explanation is suggested for the activity of the *cis*-cyclopropyl acid **23** which is also said to have auxin activity [39]. The auxin activities of phenolic compounds [40] and isatin [41] are also not readily explicable by the above theory.

CONCLUSIONS

The structural requirements for auxin activity have been tentatively defined in relation to a postulated receptor site with characteristic conformational and stereochemical requirements which are complementary to the thermodynamically most stable form of the indoleacetic acid molecule. It is argued that at least the great majority of auxin receptor sites have an absolute configuration complementary to the *S* form of α-3-indolepropionic acid. The major differences between the above receptor site theory and the charge separation theory are that stereochemical factors are taken into account and that the part of the receptor site which interacts with the indole nucleus is an area which can interact with the π electrons of aromatic rings rather than a point with a fractional negative charge.

With respect to the conformational change theory, it is suggested that the model proposed above may be preferable because (i) it offers an explanation for the anomalous activity of certain enantiomeric pairs of arylpropionic acids as well as indole*iso*butyric acid; (ii) the properties of substituents exerting effects other than, or in addition to, steric effects are accounted for, and (iii) it is unnecessary to postulate complex conformational changes once the molecule has interacted with the receptor site.

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