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STRUCTURE–ACTIVITY DIFFERENCES BETWEEN INDOLEACETIC ACID AUXINS ON PEA AND WHEAT*

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Key Word Index—*Pisum sativum*, Leguminosae, *Triticum aestivum*, Gramineae, auxins, receptor site, structure–activity

Abstract—A series of halogenated indoleacetic acids was assessed for auxin activity on pea stem and wheat coleoptile sections Activity differences between the two species were found These are discussed, in terms of differences in receptor models for pea and wheat, with the models differing in the areas covered by the 6- and 7-substituted compounds

INTRODUCTION

A common feature of the more recent theories of auxin structure–activity correlations [1–5] is that the relationship is described by reference to hypothetical receptor sites The sites are conceived as having either particular chemical, stereochemical, or charge separation properties, depending on the theory It would thus appear that recognition of the hormone by its receptors may be an important factor in the overall relationship between structure and activity It has also been proposed that our greater knowledge of animal hormone systems may offer attractive paradigms for research on the mechanism of hormone action in plants [6] It would seem reasonable, therefore, to apply accepted concepts of animal pharmacology to the auxin area Receptor models developed in this way may, in the first instance, be useful as a working hypothesis in the design of synthetic growth regulators In the longer term, it may be possible to further refine the models so that they reflect the properties of the actual receptors present in the plant To do this, the underlying assumptions used in the development of the models will need to be resolved Some ways by which this may be achieved are by the weight of consistent and adequate structure–activity data, or by binding studies with suitable compounds on isolated receptors In all these regards, the auxin activities of a series of mono- and disubstituted indoleacetic acids which have become available [7] are of interest, because the compounds are closely related to the natural hormone, and are likely to achieve their effect by a common mode of action It has also been concluded that their differing pK and lipophilic properties are not dominant factors with respect to their auxin activity [8]

The receptor site, as postulated by one of us [1] is a composite one derived from auxin activities observed in a range of species and a variety of assays It is possible that there may be activity differences between species, and a model derived for any one species may differ from the composite model As a first step in determining whether such differences may exist, structure–activity correlations for the substituted indoleacetic acids were assessed on pea [8] Further compounds are now assessed on wheat, so that comparisons could be made between a monocotyledon and a dicotyledon

RESULTS AND DISCUSSION

The activities of the indoleacetic acids are analysed by reference to the map of the composite receptor previously proposed [1] It was conceived as being complementary to the IAA molecule, and is shown in Fig 1 It was postulated that there was a region which accepted the carboxyl group, which was called the carboxyl acceptor The area corresponding to the methylene carbon of IAA was termed the α area It was further postulated that the area which accepts the indole ring was planar and electrophilic in nature, and extended beyond the boundaries of the indole ring The area is thus divided into a part which is directly covered by the indole ring (designated the Ar_1 Ar_2 area), together with surrounding areas marked a–f The area as a whole is called the electron acceptor The hatched areas correspond to areas of steric obstruction It can be seen that those parts of the site surrounding the Ar_1 Ar_2 portion do not interact with the natural agonist, yet it has been concluded that they can contribute to auxin activity if synthetic molecules overlay these areas [1] Being located next to the binding site proper, they are termed accessory areas by analogy with similar concepts in animal hormone–receptor systems [9–11]

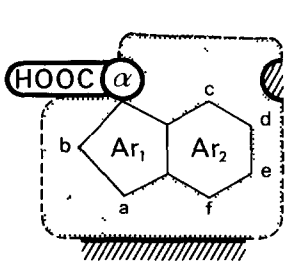

Fig 1 The composite auxin receptor site

*Part 3 in the series "Auxins" For Part 2, see ref [8]

It is assumed that the natural hormone, IAA, is an agonist for each species and can elicit a full response in each assay If this is so, and it can be seen that the carboxyl and α-methylene groups are essentially chemically and physically identical in all the compounds tested, then differences in activity may be due to differences in ability to bind to the electron acceptor Binding will be affected by steric considerations, the ability of the substituents to bind to the accessory areas, and by electronic effects of the substituents on the indole ring, which would affect the ability of that ring to bind to the Ar_1 Ar_2 area With respect to the latter electronic effects, it was previously suggested that charge distribution on molecules overlying the Ar_1 Ar_2 area may not be unimportant [1] although no firm conclusions were drawn In this regard, the recent work of Farrimond *et al* [12] is relevant These authors have demonstrated by self-consistent field molecular orbital calculations on auxins of the aryloxyacetic acid type that the charge separation hypothesis of Thimann cannot be supported, either as originally proposed [13] or in a modified form [3] The same calculations also indicate a lack of correlation between the magnitude of a fractional positive charge on the auxin nucleus and the degree of biological activity It has further been shown that a wide range of aromatic rings and halogens with differing electron densities and availability have the capacity to give rise to high auxin activity if they cover the Ar_1 Ar_2 area [1] It would thus appear that the 'charge separation' theory of auxin activity can now be regarded as obsolete It is concluded that while it may well be that charge distribution on the Ar_1 Ar_2 portion of the electron acceptor is not uniform, such non-uniformity does not appear to be a controlling factor in determining auxin activity, at least in the case of chlorine substitution In other words, the electronic effects of substituents on the aromatic rings covering the Ar_1 Ar_2 area are outweighed by their binding and steric effects

The IAA molecule is represented in Fig 2 and the way in which monosubstituted derivatives would overlay the composite site is represented in Fig 3, allowing for differences in size of the various substituents The various disubstituted derivatives would be represented by appropriate composites of these diagrams

Dose–response curves for IAA on pea and wheat are shown in Figs 4(A) and 4(B) These curves have been

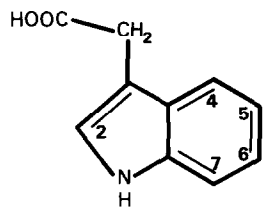

Fig 2 Indoleacetic acid

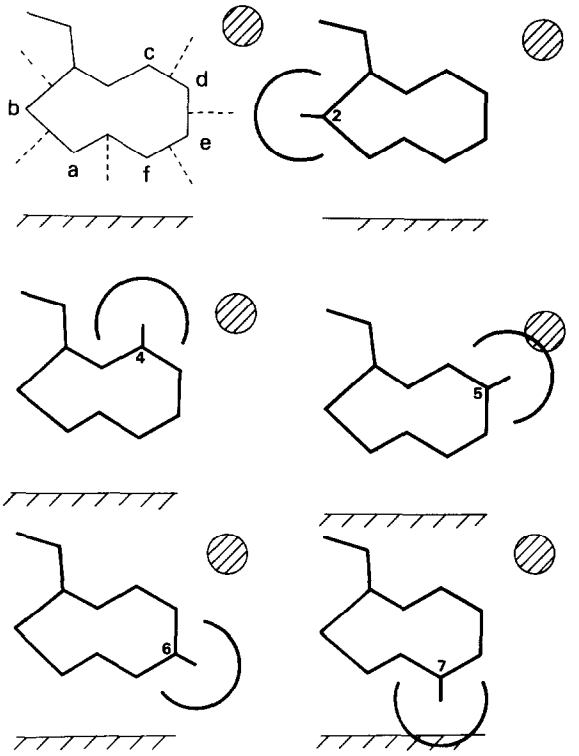

Fig 3 Overlap of monochloroindoleacetic acids with the receptor site

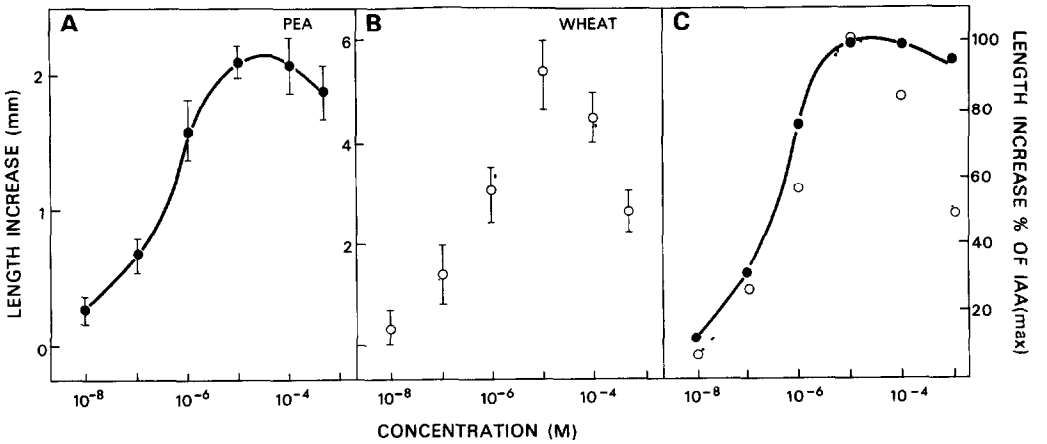

Fig 4 Comparison of IAA activity on pea and wheat Bars represent twice the standard error for a single experiment Standard errors were of similar magnitude for the other compounds

normalized so that maximum response was regarded as 100% The curves were then superimposed This is shown in Fig 4(C), and it can be seen that the curves are very similar, up to the maximum response, which is achieved at 10^{-5} M in both species Dose–response curves for the other indoleacetic acids have similarly been normalized with the parent IAA response being regarded as 100% Monosubstituted IAA's are shown in Fig 5 and disubstituted derivatives are shown in Fig 6

The capacity of compounds to elicit a response once bound to their receptors is reflected in the maximum response the compounds can achieve, and is termed the efficacy [16] The ability to bind to the site has been termed affinity [16], and is reflected in the position of the dose–response curve on the dose-axis The further the dose–response curve is to the right, the lower the ligand affinity Activities are, therefore, discussed in terms of the magnitude of the response and the concentration at which the response is achieved

The curves for 2-chloro-IAA in both pea and wheat are similar, and are also similar to the IAA curves (Fig 5 1) This compound is highly unstable, however, being easily decomposed in air [14] so that higher activities in both species are not excluded 4-Chloro-IAA also gives rise to similar curves (Fig 5 2) In both cases it appears to have higher affinity than IAA in that it reaches its maximum response at a 10-fold lower concentration In terms of the receptor site theory, the 4-chloro group would interact strongly with the 'c' area (Fig 1), and this would not be inconsistent with the activities of the benzoic acids, which are considered to overlay and bind to this area [1]

The 5-substituted indoleacetic acids also give rise to similar curves (Figs 5 3–5 5) With the larger bromine atom, however, full response is not reached until a 10-fold higher concentration than that required by IAA or the other two 5-substituted derivatives, which would imply a lower affinity This would be consistent with the proposal that the bromine atom impinges to a greater extent on an area of weak steric obstruction, as has previously been suggested [1]

6-Chloro substitution, which would overlie the unobstructed 'e' area, shows a full response in pea, but not so in wheat (Fig 5 6) In the latter species, while maximum response is reached at a lower concentration, the magnitude of the response is much lower than in the parent compound This implies that there may be a receptor difference in the 'e' area The 7-chloro compound also shows a difference, in that the compound can elicit a full response in pea, but not so in wheat (Fig 5 7) The concentration at which maximum response is reached also differs between the species, which may reflect a lower affinity in wheat The larger bromine atom gives rise to decreased response which is only achieved at a higher concentration than that of the parent molecule in both species (Fig 5 8) There thus may be receptor differences in the 'e' and 'f' areas between the species, although the difference in the 'f' area may only be slight

In the disubstituted series, it can be seen that where two substituents impinge on the obstructed 'd' and 'f' areas, as occurs in 5,7-disubstitution (Figs 6 3 and 6 4), a maximum response occurs only at high concentration With the dichloro compound, the magnitude of the maximum response is also low These results appear to be consistent with the receptor site model The 4,6-dichloro compound, however, elicits marked differences in responses between the species The receptor map show no obstructions in the 'c' and 'e' areas, on which the chlorine atoms would impinge, so that a full response would be predicted As can be seen from Fig 6 1, this is the case for pea, but not for wheat In wheat, maximum response requires a concentration of 10^{-4} M, and the magnitude of response is less than for IAA At 10^{-6} M the compound is inactive on wheat, while producing an 80% response on pea This is consistent with the proposition that there may be an area of steric obstruction in the 'e' area of the wheat receptors but not in those of pea The 4,7-dichloro

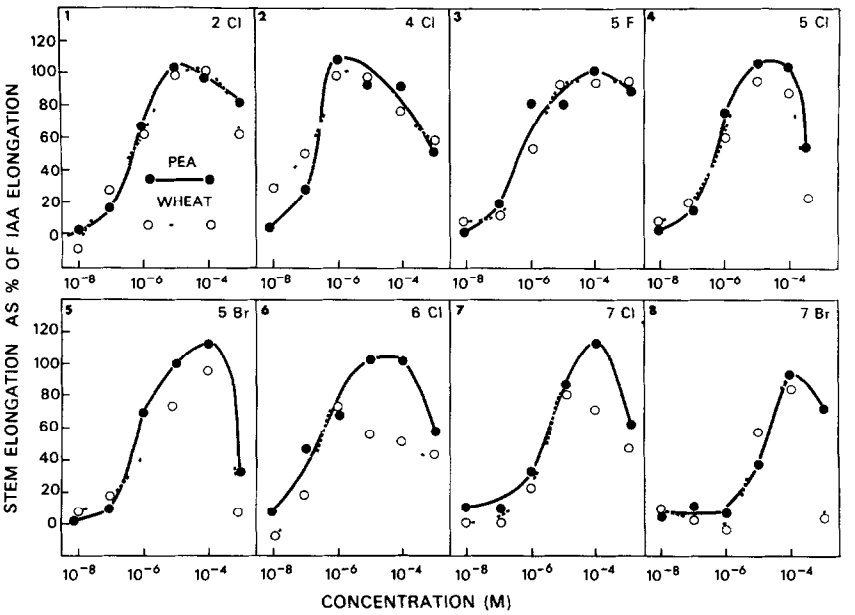

Fig 5 Dose–response curves of monosubstituted indoleacetic acids on pea and wheat (●———●) pea, (○- - -○) wheat

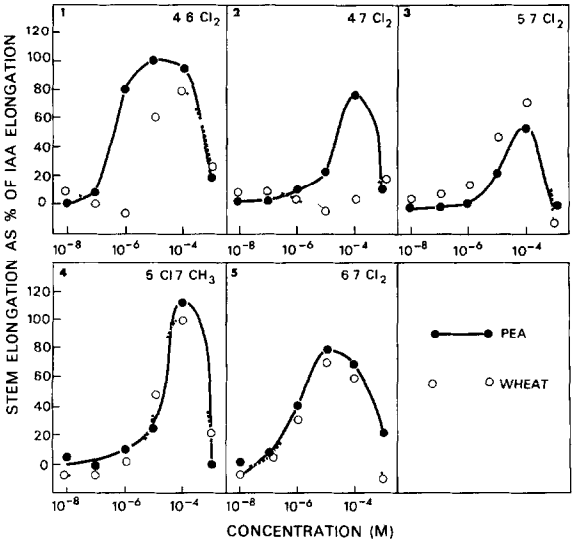

Fig 6 Dose–response curves of disubstituted indoleacetic acids on pea and wheat (●——●) pea, (O - - - O) wheat

compound also shows differences In pea an 80% response is elicited at 10^{-4} M, but the compound is almost inactive in the wheat assay (Fig 6 2) As shown, all concentrations show an essentially zero response, but in some assays, a very weak response (*ca* 10%) could be observed at 10^{-4} and 10^{-3} M This again is consistent with there being somewhat greater steric hindrance in the 'f' area of wheat Dose–response curves for 6,7-dichloro-IAA on the other hand, were found to be similar to each other, with their activity being weaker than IAA (Fig 6 5) On the model proposed above, pea might be expected to give a greater response that wheat, so that this result seems anomalous

It has previously been concluded that the structure–activity pattern of mono- and dichloro-indoleacetic acids with respect to pea is reasonably consistent with the receptor site map [8], and it would appear that the further substituted compounds tested here are also consistent with the map With respect to wheat, however, there are definite differences, and these appear to occur in the 'e' and 'f' areas, which would interact with the 6- and 7-substituted derivatives A tentative model of the wheat receptor map derived from these considerations is shown in Fig 7 It is of interest to compare these results with those of Bottger *et al* [15] who have tested the same compounds on *Avena* Comparisons are rendered difficult because generally only one value for overall activity was reported, and it has been suggested that valid comparisons can best be made only when full dose–response curves are available [8] The results obtained by Bottger *et al* are shown in Table 1

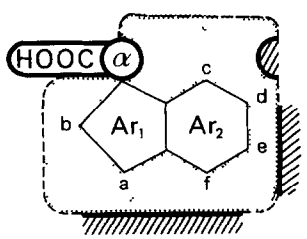

Fig 7 The auxin receptor site for wheat

Table 1 Relative activity of substituted indoleacetic acids on *Avena**

Compound	Relative activity†
IAA	1
4-Cl-IAA	10
5-Cl-IAA	1 2
6-Cl-IAA	19
7-Cl-IAA	0 13
4,6-Cl_2-IAA	0 11
4,7-Cl_2-IAA	< 0 01
5,7-Cl_2-IAA	< 0 01
6,7-Cl_2-IAA	0 11
5,Cl,7-Me-IAA	0 77
5-F-IAA	0 2
5-Br-IAA	0 3
7-Br-IAA	0 08

*Taken from Bottger *et al* [15]

†Relative activity

$$= \frac{\text{Concn of IAA giving half max elongation}}{\text{Concn of substituted IAA giving same elongation}}$$

The high activity shown by 4-chloro IAA is consistent with the activities shown here, as is the activity shown for 5-chloro-IAA The activity of 6-chloro-IAA would seem to be similar to that shown on pea rather than wheat However, wheat reaches a maximum response at 10^{-6} M compared with that reached for IAA at 10^{-5} M, and an assessment of activity based on the concentration of compound giving half the maximum elongation of IAA, would result in a value for activity higher than that of IAA, although the complete dose–response curve would indicate that the reverse is the case The comparison, therefore, may not be meaningful The activity of 4,6-dichloro-IAA appears to be similar to that of wheat rather than pea, as does the value given for 4,7-dichloro-IAA The 5,7-dichloro-IAA result is different from both, in that it is inactive on *Avena*, while giving rise to weak activity on pea and wheat The values for the remaining compounds are consistent with the values obtained here

While the construction of receptor maps can be justified as a means of expressing structure–activity correlations, whether they are representative of the various auxin receptors involved in the elongation response depends on the validity of the assumptions involved in developing the maps

The initial auxin map was derived from single values for overall activity, and there was an implicit assumption that the indoleacetic acids tested were all agonists of about equal efficacy [1] However, as can be seen from the full dose–response curves presented here, equal efficacy cannot be presumed The further possibility that some compounds may be auxin antagonists has not been developed, and will require further research On the other hand, the assumption that non-receptor factors do not exert a controlling effect may be justified to some extent This is because the non-specific processes of uptake, movement, sequestration and conjugation that are not receptor controlled will depend on the chemical and physico-chemical properties rather than size, shape and

configuration of the molecules, and it has been shown that the auxin activities of compounds of widely different chemistry can best be explained by reference to a receptor site [1] This implies that the recognition process may be significant factor, at least in cell elongation assays, although interaction with auxin-specific enzymes is not excluded

Assumptions analogous to those outlined above were used to develop structure–activity correlations for the phytotropins [17, 18] That the assumptions may be valid in the latter area is shown by the good correlation between physiological activity and capacity of the compounds to bind to the phytotropin receptor [19] It is at least possible, therefore, that the assumptions could be valid here, so that the correlations observed may in fact reflect molecular recognition by the auxin receptors

It can be seen that the structure–activity differences observed are assigned to differences in the accessory binding areas Accessory binding sites have also been postulated in animal hormone–receptor systems [9, 10] They are considered to be predominantly hydrophobic in nature, and can bind to aromatic rings [11] A similar situation would appear to exist here These sites can also differ between species as well as within species, giving rise to differing selectivities of action between antagonists [11] Since it is well known that synthetic auxins can differ in their spectrum of action, both within and between plant species, the possibility is raised that such differences may be due, at least in part, to differences in the accessory binding areas

It is concluded that there are differing structure–activity requirements for auxin action between pea and wheat, and that these differences can be expressed in terms of receptor models It is suggested that the models have value as a working hypothesis in the design of synthetic growth regulators Whether the models represent true receptor differences will depend on the resolution of the assumptions involved in their development

EXPERIMENTAL

Chemicals IAA was obtained from the Aldrich Chemical Co 2-Chloro-IAA was made by the method of ref [14] The gift of the other halogenated indoleacetic acids [7] by Dr K C Engvild is gratefully acknowledged

Pea stem section assay This was carried out as previously described [8]

Wheat coleoptile assay Seeds of *Triticum aestivum* cv Gabo were surface sterilized for 45 sec with a 4% sodium hypochlorite soln, rinsed with H_2O and left in the H_2O for 30 min to facilitate imbibition Seeds were subsequently placed on two wet filter papers in Petri dishes (10 ml H_2O, 20 seeds/dish) Plants were grown in darkness at 26° and received 30 min red light (Philips red fluorescent tube TL-40 W/15, 263 μW/cm^2 600–800 nm of which 10% was between 700 and 800 nm) 24 hr before harvesting During the last 24 hr of the growth period before harvesting, the lids of the Petri dishes were removed and plants were grown in a humid environment in total darkness at 26° Coleoptiles were harvested under laboratory lighting conditions when they were between 17 and 20 mm long Coleoptile segments 12 mm long were cut from the apical end beginning 2–3 mm from the apex After excision, the primary leaf was removed by threading each coleoptile on a glass capillary (Drummond 'Microcaps', 1 lambdas) The coleoptiles were immediately floated in citrate buffer (10^{-3} M, pH 6 5) for a period not greater than 2 hr After this, at least 10 coleoptiles were randomly distributed to each Petri dish containing 15 ml of test soln Straight growth took place in the dark at 26° and coleoptiles were measured after 24 hr to the nearest millimeter

Results are the average of at least two assays, and in each assay IAA at 10^{-5} M was included for comparison

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