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RECOGNITION OF PHYTOTROPINS BY THE RECEPTOR FOR 1-*N*-NAPHTHYLPHTHALAMIC ACID

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Key Word Index—*Zea mays*; Gramineae; *Lepidium sativum*; Cruciferae; phytotropins; 1-*N*-naphthylphthalamic acid; receptor; auxin transport inhibitors; structure–activity.

Abstract—The structure requirements for phytotropin activity and receptor binding are expressed in terms of a recognition site on the receptor with which phytotropins, including 1-*N*-naphthylphthalamic acid, interact. It is postulated that the site can be represented by a large region which accepts planar molecules, and is possibly electrophilic in nature. A second area is also postulated which may be lipophilic or electrophilic, together with a carboxyl acceptor. It is suggested that if the requirements of the carboxyl acceptor and adjacent area are met, then phytotropin activity will result if the candidate molecule has a configuration, or can adopt a configuration, such that a conjugated portion of the molecule can interact with the larger area. It is argued that the close relationship observed between receptor binding and effect on the gravitropic response implies that the receptors may be directly involved in the gravitropic response mechanism.

INTRODUCTION

The phytotropins are a class of compounds with common chemical and stereochemical properties [1], and which act by a common mode of action [2]. They are known to bind to a site or sites present in maize coleoptile membranes [3] and the entity on which this site(s) exists fulfils the requirements of a receptor [3–6]. The compounds have been classed as 'auxin transport inhibitors' because they can prevent the active polar movement of auxin. However, this term may be misleading to the extent that it is regarded as the mechanistic property which explains all their various physiological effects. Firstly, it may be an inappropriate description of their action at the cellular level, when the receptors of only a few cells are engaged by them, and secondly, as is known in animals [7], receptors may perform different functions in different parts of a given organism. It is thus possible that these receptors may have a physiologically significant role to play in directly controlling other plant growth functions besides auxin transport. In support of this possibility, there is evidence to suggest that the ability of phytotropins to inhibit auxin transport, without more, may not be enough to explain some of their physiological effects [8], in that differential growth can be induced by them under conditions where auxin movement is prevented. The properties of the receptor are therefore of some interest.

One property of intrinsic receptors is that they have characteristics to enable them to recognize the presumed endogenous ligand (by definition, an agonist) with which they interact. These recognition characteristics are reflected in the properties of molecules, natural and synthetic, which bind to the recognition site [9]. Since there is also a relationship between binding and physiological effect, and such a relationship has been observed here [3], it was considered possible that if the relationship could be defined more accurately, it might assist in determining the nature of the action of the receptor, as well as the mechanisms of those physiological processes that phytotropins are known to affect.

In animal pharmacology, it is known that a receptor may have more than one recognition site, if only because agonists, which produce a response, and competitive antagonists, which prevent that response, are recognized differently [7]. With the present receptor, not only do the phytotropins bind, so also do the morphactins **7** [10], which appear to be chemically and stereochemically different. 2,3,5-Triiodobenzoic acid (TIBA) **8** is now known to bind very weakly [11], and does not come within the structure–activity rules as so far established for the phytotropins. With respect to 1- and 2-naphthylphthalamic acids (NPA) and TIBA, it has been suggested that there may be two different sites for the compounds on the same complex [11]. It is thus possible that there may be more than one recognition site on the one receptor macromolecule, as is known to occur in animals. A solubilized form of the receptor also binds to indoleacetic acid (IAA) **9** and its synthetic analogues [12], although this binding may not be physiologically significant [3, 12]. As a preliminary step in determining the relationship between these differing sites, the recognition site examined here will be that for the phytotropins only.

It was suggested earlier that binding-activity relations for these compounds might more appropriately be expressed in terms of a model, rather than in terms of rules [8]. Sets of closely related molecules were therefore examined to determine the nature of the relationship between them and the receptor, and the relationship so derived was used to further refine a provisional model which may be regarded as representing the recognition site. The model so derived is correlated with the structure–activity rules.

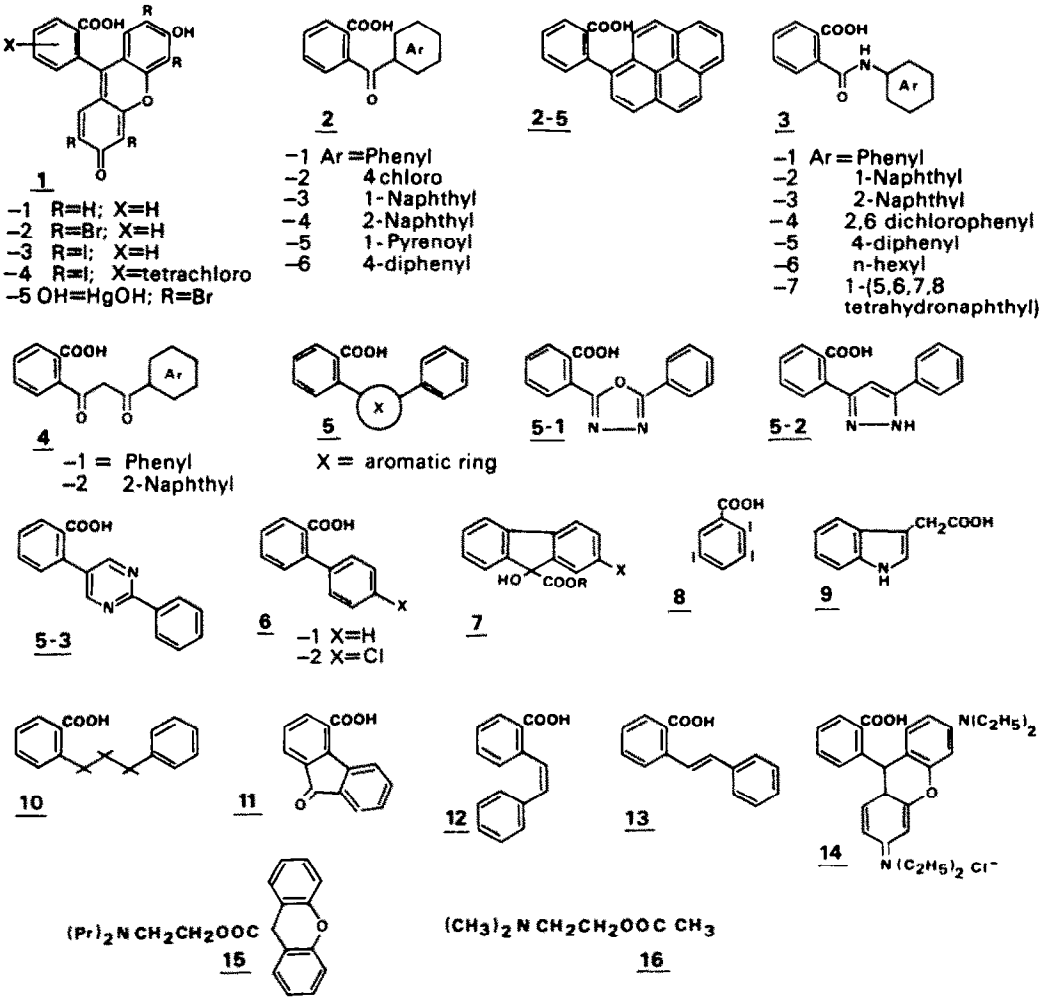
DISCUSSION

The structure–activity rules for phytotropins were developed initially through their ability to abolish the gravitropic response in cress roots [13]. This was later extended to their auxin transport inhibiting properties [2]. The rules are that active compounds should possess:

1. A carboxylic acid function (or one which can become available by hydrolysis) which is attached to;
2. An aromatic ring, which is connected at the *ortho* position to;
3. A second aromatic ring;
4. The aromatic rings may be separated by a conjugated or planar system of atoms;
5. There is also a spatial requirement which was expressed by saying that high activity will be reached when the distance between the centres of the two extreme aromatic rings was at least 7.3 Å [13]. Some examples of the differing chemical types of compound which conform to the rules are shown by structures 1–6.

The rules so developed were based on gravitropic activity rather than receptor binding. Development of a model based on gravitropic activity would be based on an assumption that either the compounds are all agonists of about equal efficacy (capacity to elicit a response once bound to the receptor), or they are competitive antagonists of differing affinities [3], to the extent that such concepts can be applied to plants. However, while a relationship between binding and activity has been observed for compounds so far tested, there are some discrepancies [3]. The NPA molecule, for example was found to be 30–100-fold less active in bioassays than its binding ability indicated. This could be due to non-receptor factors (see below), to efficacy differences between the differing types of molecule or to species differences. Molecules were therefore classified as to chemical types, and binding data are presented in this way. The results obtained are correlated with the ability to abolish the gravitropic response in cress roots so that the relationship between receptor binding and biological response can be seen. Cress was chosen to assess the gravitropic response because this was the species used to develop the structure–activity rules. While phytotropins can abolish the gravitropic response in maize [14, 15] (as well as in other species [16–18]), assessment of maize is both experimentally more cumbersome, and complicated by the occurrence of nostic curvature, which is not a problem in cress.

For the aryl and aroyl benzoic acids (Fig. 3), there is a good correlation between binding and activity, and a strong correlation has been observed for the arylphthalamic acids (Fig. 4) [8]. The fluoresceins are shown in



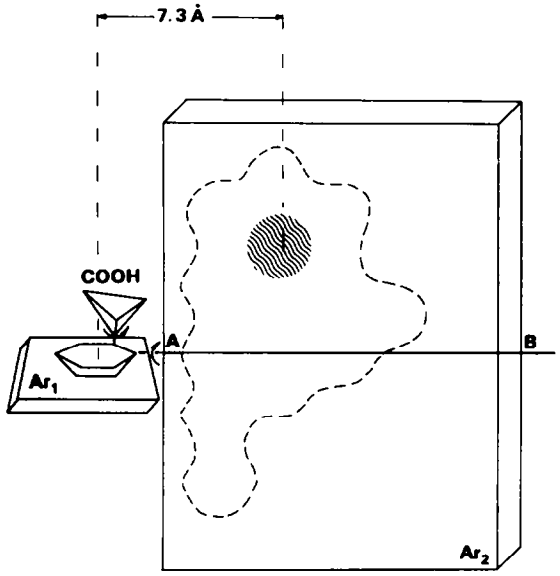


Fig. 1. Model of the recognition site.

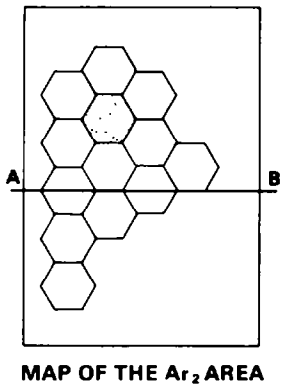


Fig. 2. Map of the Ar_2 area of the recognition site.

Fig. 5, and again there is a relationship between binding and activity. Comparisons between the sets of results show systematic differences between the types. In particular, the phthalamic acids are less active for a given degree of binding than the aryl and aroyl benzoic acids. It is concluded that while binding affinities are essential to determine the receptor recognition characteristics, the biological data can be relied on to some extent if comparisons are made from within the chemical types.

A model of the recognition site is shown in Fig. 1. The required carboxyl group [3] would interact with the carboxyl acceptor, designated COOH, an aromatic ring attached to the carboxyl binds to an area designated Ar_1 while the remainder of the molecule, including the second aromatic ring, would overlay a second area (Ar_2) area with at least some atoms being able to bind to it.

The Ar_1 and Ar_2 regions are postulated as areas, although other structures, e.g. a slot or envelope, are not excluded for either region. The shaded area is postulated as a sensitive area, which, if overlain by a molecule which

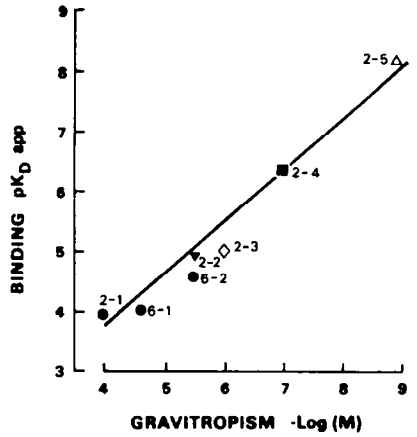


Fig. 3. Binding/gravitropic activity of aryl and aroyl benzoic acids. pK_D app: molar concentration (K_D app) giving 50% reduction of specific [^{3}H]NPA binding expressed as its negative logarithm. Gravitropism: lowest concentration at which root gravitropic response is abolished.

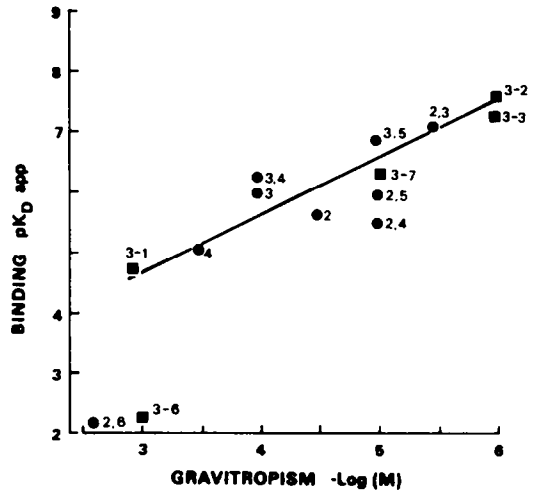


Fig. 4. Binding/gravitropic activity of aryl phthalamic acids. Values defined as in Fig. 3. ■ Numbers refer to chemical structures; ● numbers refer to chlorine substitution pattern on compound 3-1.

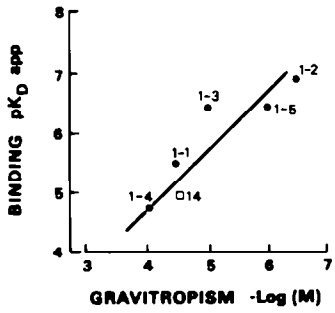


Fig. 5. Binding/gravitropic activity of fluoresceins. Values defined as in Fig. 3.

otherwise fulfils the requirements, will give rise to higher binding than if the molecule impinged elsewhere. The distance requirement of 7.3 Å as expressed in the rules, corresponds to the distance between the centre of the shaded area and the centre of the area where a benzene ring would impinge on Ar_1. The area within the dotted shape represents the area within which the compounds considered here can impinge and give rise to receptor binding. Configuration, conformation and conformational change are described in terms of the two degrees of freedom of the model: rotation about carboxyl/Ar_1 and rotation about Ar_1–Ar_2 as shown by the arrows. The line AB is there for the purpose of showing symmetry of the site. Figure 2 can be regarded as a map of the Ar_2 area, with the hexagons drawn being used to identify those parts of the site on which the various molecules impinge. The perimeter of the hexagons is represented by the dotted line in Fig. 1. The way various molecules are envisaged to engage the site is shown in Diagram 1. The benzoic acid moiety binds to Ar_1 and the carboxyl acceptor. This fixes the molecule, and determines the way in which the remainder of the molecule engages Ar_2. The extended conformation shown is postulated because it would be obtainable by the active molecules [18].

The carboxyl acceptor

A carboxylic acid is required for biological activity [13] and compounds lacking a carboxylic acid, but fulfilling the other requirements for biological activity, do not bind [3]. Whether other acidic or isosteric groups can replace the carboxyl has not been investigated. A carboxyl acceptor is therefore postulated.

The Ar_1 region

All highly active compounds which also bind strongly that have been assessed so far have a benzene ring which could interact in this region. Since receptor binding is reversible, the nature of the interaction could be either lipophilic, or possibly a π-bonded or charge-transfer complex which is non-covalent. Analogues of the phthalamic acids which do not have an aromatic ring to engage this region are either inactive, or have only low activity at best [16, 18]. Binding of some kind is therefore probably required, and it may have an electrophilic element. Substituents around the ring do not increase gravitropic activity [13, 16, 19], and it can be seen that the tetrachloro substituted compound **1–4** (Rose Bengal) binds less and is less active than its unsubstituted analogue **1–3** (Fig. 5), although steric effects may be a factor here (see below). Further data will be required before firm conclusions can be drawn.

The Ar_2 area

Subject to any size, shape and conformational requirements imposed by the site, phytotropin binding and activity will result if an interacting molecule can overlie this area. The degree of activity will depend on the ability to bind to Ar_2, which in turn will depend on the size of the appropriate portion of the interacting molecule. As with

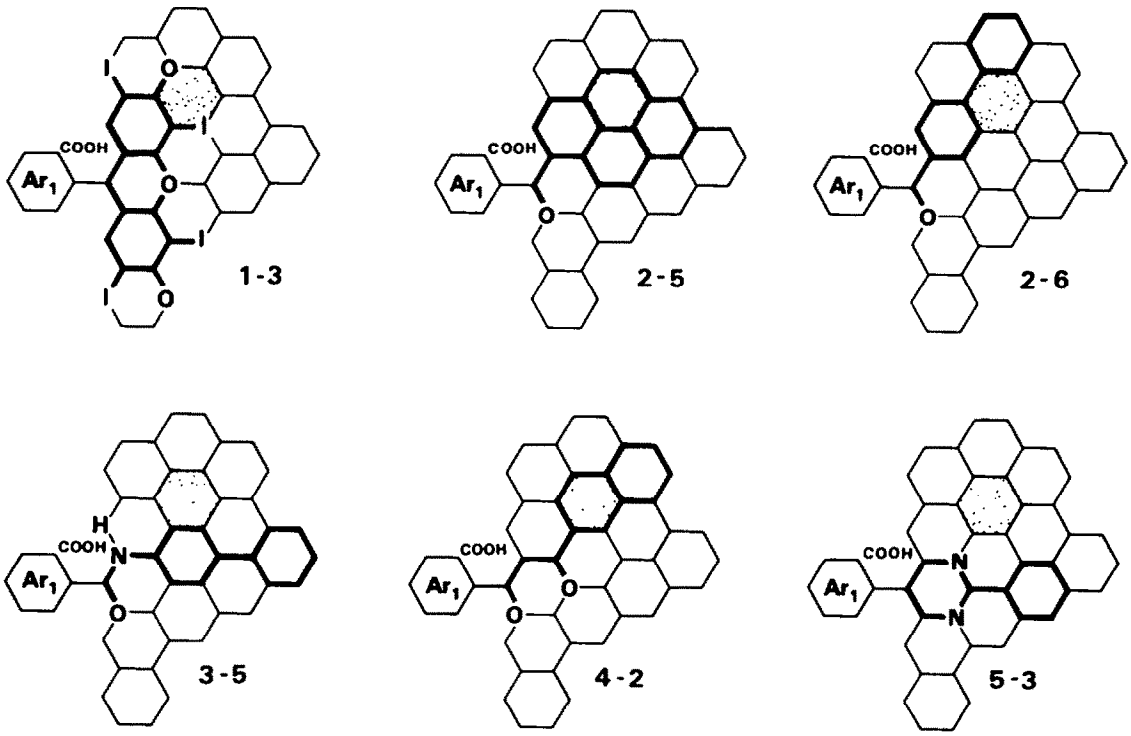


Diagram 1. Overlay of active molecules on the recognition site. **1–3** and **2–5** are active both in binding and on the gravitropic response; **2–6** [17], **3–5** [16], **4–2** [33] and **5–3** [34] are gravitropically active at $\sim 10^{-6}$, 10^{-5}, 10^{-8} and 10^{-6}, respectively.

the Ar_1 area, binding could be lipophilic and/or electrophilic in nature. In the phthalamic acid series, the binding of the *n*-hexyl compounds **3–6** is too weak to assess, while the phenyl compound **3–1**, which would be less lipophilic, has significant binding (Fig. 4). Also, the naphthyl compounds **3–2** and **3–3** bind very strongly, while the tetrahydronaphthyl analogue **3–7** which is less aromatic, binds more weakly (Fig. 4).

Calculated lipophilicities of these latter and other molecules indicate that lipophilicity does not appear to be systematically related to binding [20]. Selected values are shown in Table 1 [20]. It is suggested, therefore, that the nature of the binding may include an electrophilic element, perhaps a type of π-bonded or charge-transfer complex. Such binding would be analogous to the similar binding postulated for the auxin receptor [21]. However, hydrophobicity and electronic effects of substituents are not excluded as possible factors, and remain to be more accurately assessed. In the aryl and aroyl benzoic acids, as well as the phthalamic acids, it can be seen that binding increases with the area covered (Figs 3 and 4). In the latter, an unsubstituted benzene ring binds less strongly and is less active than the monochloro substituted analogues, which in turn are less effective than the dichloro substituted compounds. Chlorine substitution in both the phenyl and benzoyl benzoic acids (Fig. 3) increases both binding and activity, while substitution of naphthalene for phenyl increases activity as well as binding in both the phthalamic and aroyl benzoic acids. Substitution of pyrene for benzene as in the aroyl benzoics **2** gives rise to the most active phytotropin known: 2-(1-pyrenoyl) benzoic acid (PBA) **2–5** [22]. This molecule has four fused benzene rings which can overlie the area, as shown in Diagram 1. It is therefore postulated that increased coverage of the Ar_2 by atoms which can bind to it gives rise to both increased binding and activity.

Stereochemical analysis

A shape requirement is an essential element in the recognition characteristics of a receptor [7]. The requirements for gravitropic activity were shown to include a requirement for conjugation or planarity, but this requirement was not completely defined [13], although some limited conclusions have been drawn [23]. In terms of receptor binding and the receptor model, the requirement can be seen as having three different aspects. Firstly, there is the size, shape and planarity of the region of the molecule which binds to Ar_2; secondly, the relationship between Ar_1 and Ar_2, and thirdly, the relationship between the carboxyl and Ar_1. The model proposed therefore renders the shape requirement more amenable to analysis. A fourth aspect, the symmetry of the site, is also disclosed, together with the possibility that the Ar_2 region need not be uniform.

Table 1. Binding affinities and calculated octanol/water partition coefficients (log *P*)

Compound	pK_D app	Log *P**
3–2	7.60	2.79
3–6	< 4	2.47
4–1	7.20	1.80
5–2	7.47	4.26

*Ref. [20].

The model shown in Fig. 1 is not symmetrical. It can be seen that if Ar_1 is fixed, then regardless of its configuration with respect to Ar_2, most molecules coud interact with Ar_2 in the area above the line AB, or below it, but not both at the same time. This is shown for a type 5 molecule in Diagram 2. There are thus three possibilities for the absolute configuration of the receptor: (1) as shown, with binding essentially above AB; (2) binding below AB (which would be the mirror image of 1) or (3) Ar_2 is symmetrical with respect to AB. The fluoresceins are symmetrical with respect to Ar_2 and they can bind (Diagram 1; **1–3**). Ar_2 is therefore either symmetrical with respect to the area covered by the fluoresceins, or, if not, then the site would seem not to offer steric hindrance on the alternate side where the fluoresceins must impinge. Other than this, the data do not distinguish between the possibilities. The absolute configuration in Fig. 1 is thus arbitrary. Subsequent conclusions with respect to stereochemistry are subject to this qualification.

It was considered possible that the Ar_2 region need not be uniform [8]. Type 5 compounds can be highly active [1, 2, 13], including those with a 5-membered central heterocyclic ring, including 5-(2′-carboxyphenyl)-3-phenyl pyrazole (CPP) **5–2**. It has been concluded that the outer ring is essential for high activity [13], and it can be postulated that for binding the ring must impinge on a sensitive area which can bind more strongly to molecules than the remainder of the region. Such binding is reflected in higher activity. Because of the requirement for planarity (discussed below) type 5 compounds can only adopt two configurations with respect to Ar_2, as shown by 5a and 5b in Diagram 2. This limits the regions where the sensitive area [8] may be. The position of the sensitive area is therefore proposed as shown.

The more active phytotropins have aromatic rings or atoms which are also capable of overlying the shaded sensitive area. These include the chemically different structures **1–6**. For the phenylphthalamic acids, the way in which these compounds could cover Ar_2 is shown in Diagram 3. The 2-chlorophenyl compound binds more strongly than the electronically equivalent 4-substituted compound (Fig. 4). If the 2-chloro molecule must adopt a planar configuration in the Ar_2 area, it is more likely to adopt the configuration whereby the 2-chloro would impinge on the sensitive area as drawn, rather than the alternative conformation, because of greater steric hindrance due to the amide carboxyl as compared with the amide hydrogen [23] (Fig. 6). The 3-chloro compound which can adopt two planar conformations, one of which would partly overlie the sensitive area, also binds more strongly than the 4-chloro compound, which cannot. In the disubstituted compounds, the 2,3-dichloro analogue, where both chlorines would overlie the area, binds more strongly than the electronically equivalent 2,5-compound, where only one chlorine can bind to the sensitive area. A sensitive area would seem the most tenable explanation for the differences in binding ability of these latter two compounds [8]. A similar phenomenon is observable in the naphthylbenzoic acids, where the 2-naphthyl compound **2–4** binds more strongly than the 1-naphthyl **2–3** (Fig. 3), yet the area of Ar_2 covered is the same, and the naphthyl rings are of course identical. It can be seen from Diagram 4, **2–3** and **2–4**, that the second ring of the 2-

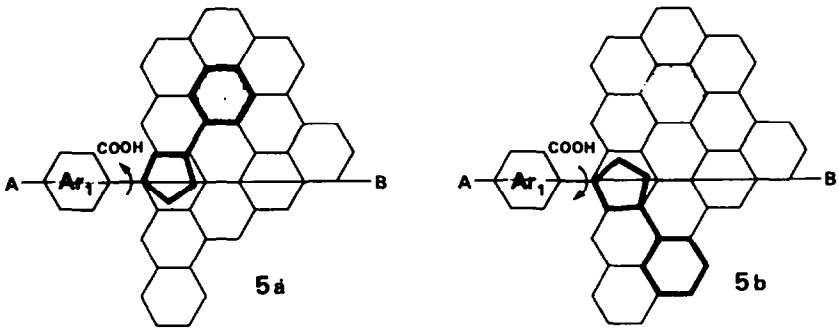


Diagram 2. Overlay of a Type 5 molecule on the recognition site.

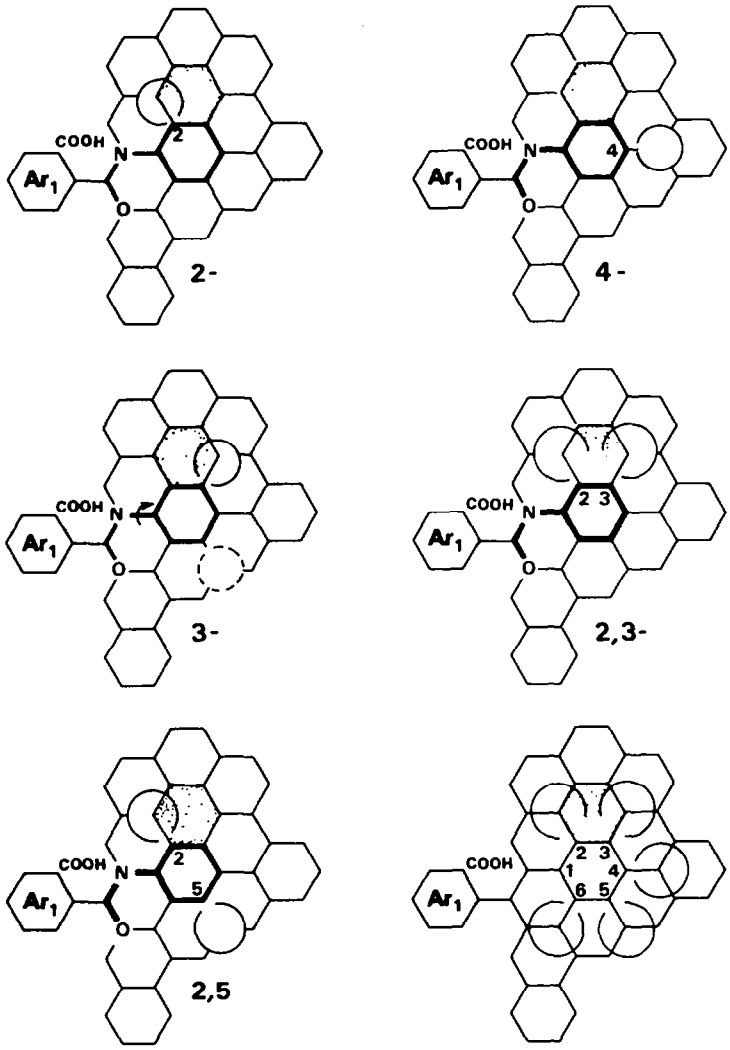


Diagram 3. Overlay of chlorophenylphthalamic acids on the recognition site.

naphthyl compound could overlie the sensitive area, while that of the 1-naphthyl would not. On this basis, it might be predicted that 1-NPA 3–2 should bind more strongly than the 2-analogue 3–3 because the latter could not overlie the sensitive area Diagram 4. However, the binding of the two compounds is essentially the same. This anomaly may be due to steric factors, and is discussed below.

It is concluded that a sensitive area exists which, if it is engaged by a molecule capable of binding to the receptor as a whole, will give rise to enhanced binding. The position

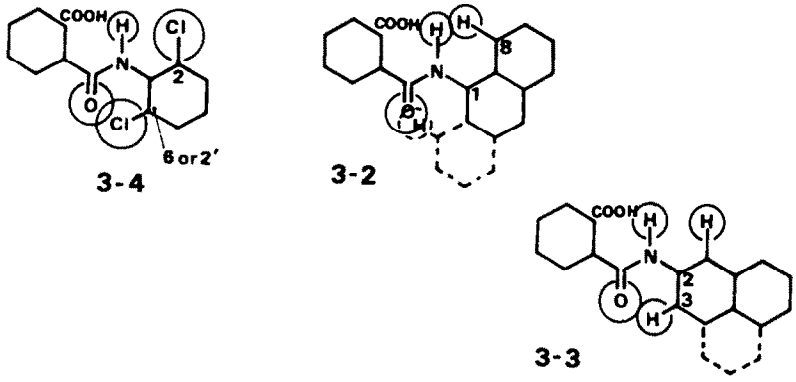


Fig. 6. Steric hindrance in the arylphthalamic acids.

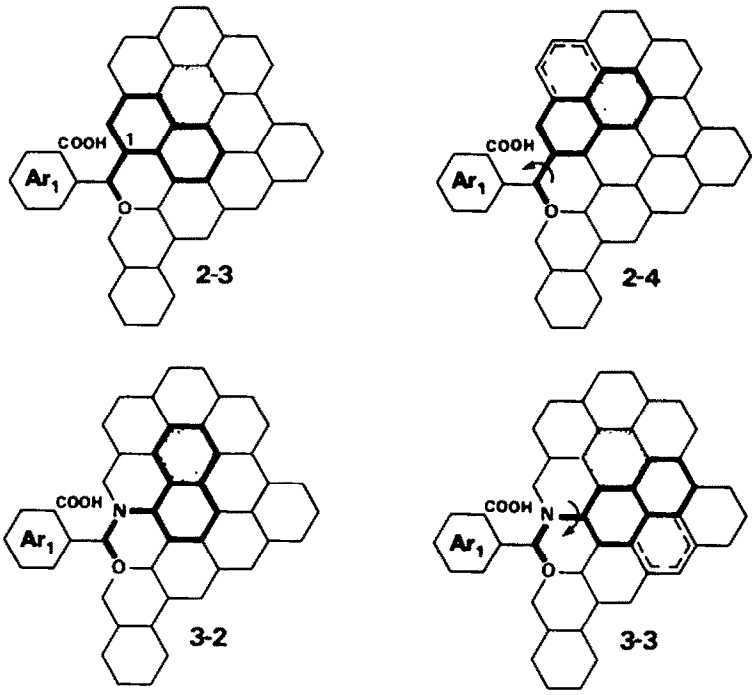


Diagram 4. Overlay of naphthylbenzoic and naphthylphthalamic acids on the recognition site.

of the sensitive area is shown as a shaded area on the model (Fig. 1).

Diagram 2 shows coverage of the Ar_2 area by selected molecules. The composite coverage of these molecules includes the whole of the area delimited by the hexagon grid. Since all these molecules are active with respect to gravitropism, it is suggested that the Ar_2 area may be at least as large as that shown, and it is possible that it may be larger. This aspect is as yet unexplored. While such a large and comparatively non-specific area may seem unlikely for a receptor that is presumably there to select and recognize a specific endogenous ligand, there are interesting parallels in animal pharmacology to justify the postulate. It is known that cholinergic, histaminic, α-adrenergic and serotonin competitive antagonists often have two, and in some cases three, adjacent or fused ring structures which are essential for high activity [7]. By way of example, propantheline **15** is a competitive antagonist for acetyl choline **16** [7]. These parallels raise the speculative possibility that phytotropins may be antagonists.

Molecules which can only have a planar structure where they would overlie the Ar_2 area can be active [23]. The fluoresceins **1** and the aryl benzoics **6** are examples of this type of molecule [24], and their activity is reflected in their ability to bind. It has been shown that other molecules with high physiological activity and binding affinity have structures which are either planar or capable of becoming planar in this area [3, 23]. The Ar_2 area is therefore capable of accepting planar molecules. There are also many molecules which are not conjugated in the appropriate region, yet have activity, although such activity is weak [1, 13].

The conformationally mobile diaryl propane **10** is an

example of this, and exhibits a low activity with respect to the gravitropic response [25]. An explanation in terms of the present model is that compounds with an aromatic ring which can overlie Ar_2, and that ring can be positioned so as to bind, will have activity, even if non-conjugated atoms, which may only bind weakly, are present. The degree of binding would depend on both the ability to adopt the correct conformation, and the binding area of the aromatic ring. Information as to the extent to which the Ar_2 area can accept chemicals which are non-planar in this area can be obtained from the examination of sterically constrained molecules. The aryl phthalamic acids have stereochemical properties which are relevant to this region. While most of these compounds can both bind and have physiological activity, 2,6-dichlorophenylphthalamic acid **3–4** is exceptional in that it is inactive gravitropically and does not bind, yet it has a π-bonded structure in the Ar_2 area, and would be electronically equivalent to the 2,4-dichlorophenyl analogue **3–5**, which both binds and is active. It was suggested that the reason for inactivity was a conformational one, in that the *ortho* chlorine groups would prevent the aromatic ring and amide group from becoming coplanar or nearly so, because of steric hindrance [23]. The configurations adopted by molecules in the crystal state can be used as a guide to the conformations they may adopt in solution [26–28]. That the preferred conformation in the crystal state is not planar for the Ar_2 area is shown in Fig. 7 and is consistent with the above proposal. The angle the 2,6-dichlorophenyl moiety makes with respect to the amide is some 45° less than the phenyl and naphthyl analogues: compare **3–4** with **3–3** and **3–1** (Table 2; last column). Resistance to change of conformation in this area would also be high, because the chlorine atoms would impinge on both the amide oxygen and hydrogen [27] (Fig. 6). In the active naphthyl and phenyl analogues **3–1** and **3–3**, the rings are out-of-plane with the amide by about 20° and 10° respectively in the crystal state (Table 2: last column) [28]. If these are their favoured conformations in solution, then either the Ar_2 region can accept molecules of these shapes as well as molecules planar in this area, or the degree of binding is sufficient to overcome the resistance to conformational change. Such resistance would be provided by interference between the amide oxygen and the hydrogen on carbon-3 as in **3–3** (Fig. 6). In the case of 1-NPA, **3–2** additional resistance would be provided by interference between the amide hydrogen and the carbon-8 hydrogen (Fig. 6). It has been suggested that it may adopt a conformation similar to the 2,6-dichloro compound for these reasons [28], yet 1-NPA binds to the receptor. However, the resistance to adopting a planar conformation in the Ar_2 region would be much less than the 2,6-dichloro compound, and may result only in reduced binding. As shown above, the binding of 1-NPA is about the same as 2-NPA, yet it would be predicted to be greater, because it should overlie the sensitive area. The observed results for these two compounds are thus in qualitative agreement with the above explanation.

It is concluded that if a molecule has high resistance to adopting an essentially planar conformation in that part

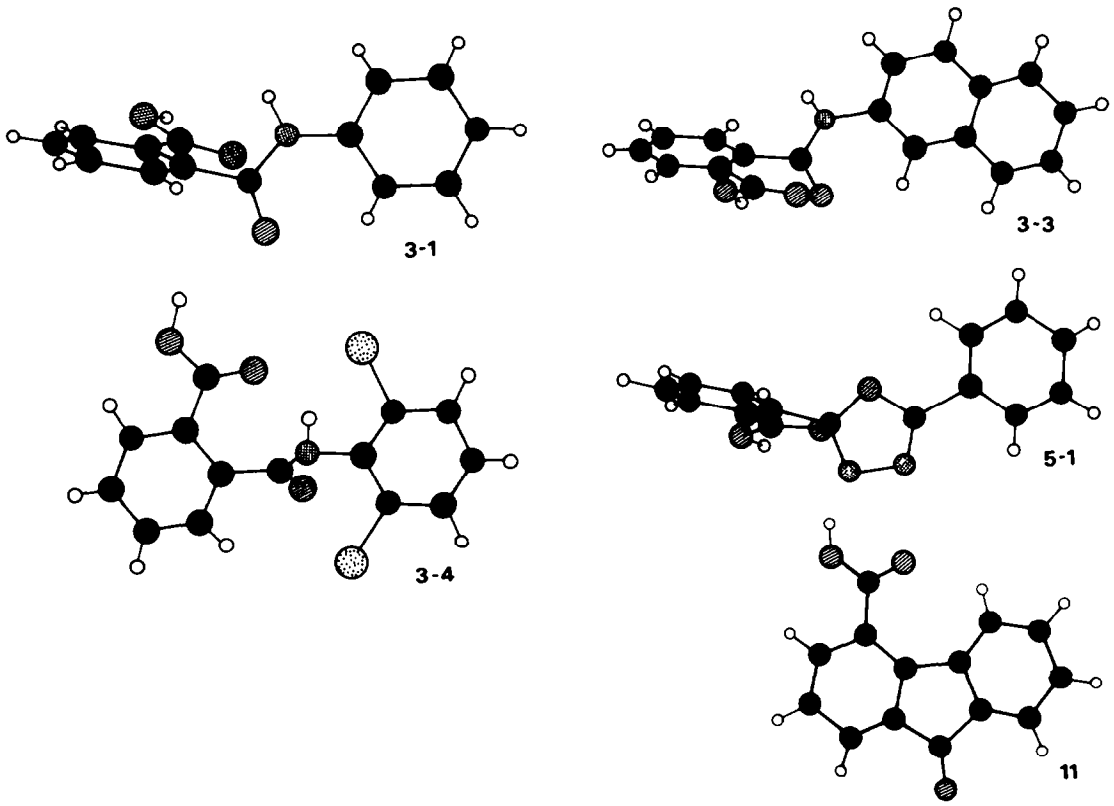


Fig. 7. Molecular conformations in the crystal state. **3–1**, **3–3** and **5–1** derived from ref. [26]; **3–4**, [26]; **3–4**, [27]; **11**, [28].

Table 2. Torsion angles of molecules in the crystal state

Compounds	Torsion angles		
	Ar_1–COOH	Ar_1–Ar_2	Within Ar_2
3–1*	3.8, −10.7	−82.3, −90.4	−51.7, −22.4
3–3*	7.2	88.0	19.2
3–4†	2.8	77.1	66.9
5–1*	7	90	11.3
11‡	26	0	0

*Taken from ref. [26].
†Ref. [27].
‡Ref. [28].
Angles measured are those related to the torsion angles of the model in Fig. 1.

which would overlay the Ar_2 area, then it will not bind to the receptor, although the degree of steric resistance necessary remains to be quantified.

The crystal structures show that **3–1**, **3–3** and **5–1** have conformations in this region which would interact with a receptor having Ar_1 and Ar_2 essentially at right angles (Fig. 7 and Table 2; centre column). Ar_1 and Ar_2 are therefore provisionally placed at an angle to each other in the model. In the fluorenone **11**, the portions of the molecule which would overlay Ar_1 and Ar_2 must be coplanar (Table 2; Fig. 7) and this molecule shows very little activity. It is, however, a very short molecule, and would have only low activity at best [23]. No firm conclusions can therefore be drawn from its configuration [23]. It can be concluded, however, that molecules which favour an out-of-plane conformation with respect to Ar_1–Ar_2 can bind to the receptor.

The active molecules analysed in Fig. 7 and Table 2, first column, have their carboxyl almost in plane with the aromatic ring to which they are attached. The fluorenone **11**, where steric hindrance would be greater because of coplanarity of the two aromatic rings, has the carboxyl only 26° from the plane. On the other hand, Rose Bengal **1–3**, which has large groups on either side of the carboxyl which would tend to make the planar from less likely, retains activity. No firm conclusions as to what may be the interacting conformation can be drawn on the data available, therefore.

Other factors

While the model is consistent with the binding data, it would appear to be inconsistent with the *in vivo* activity of the *cis* stilbenes **12**, which are nearly as active as their *trans* analogues **13** at least with respect to the gravitropic responses [25], because for steric reasons, a planar configuration cannot be achieved unless isomerization occurs.

The model assumes that the most stable conformations in the crystal state are the interacting conformations, so that the conformation shown is provisional only. Firstly, the preferred conformations of molecules in solution may be different from those in the crystal state, while secondly, other interacting conformations are possible if the energy barriers are low, and less than the binding affinity of the receptor. There is also no reason why the recognition site should adopt the predominant conformation of the ligand in solution. In some cases where the natural ligand is known, binding can still occur even though a conformation of low population frequency is involved [29].

The postulated model is of the static lock-in-key type, while agonist properties may require a conformational change to activate the receptor macromolecule in order that an effect may be produced [7]. That is, there may also be a dynamic aspect. If this is so, then the model again requires further development. The value of the model, therefore, lies in its ability to suggest what the nature of such conformational requirements may be, so that they can be assessed with appropriate model compounds and more quantitative estimations of conformational barriers by computer modelling techniques.

As shown above, there are significant differences in biological activity and binding ability between the chemical types. Again, no firm conclusion can be drawn on the data available as to the reasons for this, if only because non-receptor factors could be involved. For example, a plausible explanation for the lower gravitropic activities of the arylphthalamic acids than might be expected from their binding affinities is that they are amides, and would be subject to hydrolysis to inactive products *in vivo* but not *in vitro* [3]. In this regard, it is possible to speculate that the hydrolysis rates of the 2-chloro-substituted compounds would be less than those without a 2-substituent because of steric interference. They might therefore have a relatively higher *in vivo* activity. As can be seen in Fig. 4, this appears to be the case.

A good correlation between binding affinity and gravitropic activity within the chemical types, even though the species used to assess these properties are different, has previously been found [3]. This is now confirmed and further defined. The correlation holds through stereochemical as well as chemical parameters, which means that there is a recognition process with respect to these molecules in cress as well as in maize. The concentrations of chemical which saturate the NPA receptor in maize are also of the same order of magnitude as those which abolish the gravitropic response in cress. There is thus strong evidence that the NPA receptor exists in cress, and it is at least very similar in its recognition characteristics to that in maize.

CONCLUSIONS

The binding affinities of differing types of phytotropins have been used to construct a provisional model of their receptor recognition site. The model so derived may have value as a working hypothesis for further defining the physical, chemical and stereochemical parameters of the site, and thus assist in the elucidation of the role and function of the macromolecule on which the site exists.

The close similarity between the receptor present in maize and those in cress would seem unlikely to be fortuitous, and implies an evolutionary pressure to maintain the recognition characteristic, and thus the receptor. In other words, the receptors may exist to perform an essential physiological purpose or purposes. This prompts the speculation that the receptors may be directly involved in the control of the plant's response to gravity. Since there is no satisfactory proposal which can explain the root gravitropic response mechanism [30], and since none of the proposals either incorporate or make allowance for the presence of these receptors, then it is suggested that the possibility that they may form part of the mechanism at least warrants consideration.

EXPERIMENTAL

Plant material for binding assay. For consistency, only one cultivar of maize (*Zea mays* L. cv PX-82) was used in all experiments, so that close comparisons of binding affinities could be made. Where compounds had been previously assessed using a different cultivar (not available to us) [3] values obtained were similar, except for PBA, which was found to bind more strongly here. Seeds were surface sterilized with a 4% solution of sodium hypochlorite for 45 sec, followed by rinsing in distilled water. Prior to germination seeds were soaked in distilled water for at least 12 hr to facilitate imbibition. Germination took place in darkness at 25° in plastic trays containing a mixture of perlite and vermiculite (1:1). After 5–6 days, coleoptiles were excised, the primary leaves removed from the coleoptiles and kept chilled on ice. These and subsequent procedures were performed in daylight.

Preparation of binding fractions. In general, the methods previously described by Batt *et al.* [31] and Ray *et al.* [32] were followed. The coleoptile membrane preparation was performed at 0–4°. The tissue (usually 7–12 g/assay) was cut into small pieces with a cold razor blade and homogenized, by pestle and mortar, in an equal volume of grinding buffer (0.25 M sucrose, 50 mM Tris; 1 mM disodium EDTA; 0.1 mM $MgCl_2$, adjusted with acetic acid to pH 8.0). The homogenate was squeezed through 20 μm nylon cloth and the residue reground and extracted twice more in equal volumes of the buffer. The combined extracts were centrifuged at 4000 *g* for 20 min and the pellets discarded. The supernatant was recentrifuged for 30 min at 38000 *g*.

Binding assay. The membrane pellet was resuspended in binding medium (0.25 M sucrose, 10 mM trisodium citrate, 5 mM $MgSO_4$ adjusted with acetic acid to pH 5.5) using a Teflon–glass homogenizer. To each ml of binding fraction 20 μl of [^{3}H]NPA (5×10^{-7} M) was added to give a final concentration of 10^{-9} M. The radiolabelled binding fraction was divided in 3 ml aliquots to which 30 μl of ethanolic NPA or the test compounds were added at various concentrations. Control samples were obtained by the addition of 30 μl ethanol. Triplicate 0.9 ml samples were taken from each aliquot and placed in 1 ml polycarbonate tubes. All manipulations were carried out at 0–4°C. After 30 min (the time shown to be necessary for the NPA-NPA or compound binding site complex to reach equilibrium, [5]). The tubes were centrifuged for 45 min at 38000 *g* (Beckman fixed angle rotor TYPE-25 rotor). The supernatants were decanted and the surfaces of the pellets gently washed with 1000 μl distilled water. The membrane pellets were allowed to dissolve overnight in 200 μl 10 mM Tris. The pellets were recovered using 3 × 200 μl aliquots of 10 mM Tris, transferred to scintillation vials and counted in 10 mls of scintillation fluid (4 *g* 2,5-diphenyl-oxazole/l. toluene, mixed 2:1 with Triton X-100). Total radioactivity was measured, using a Beckman LS 6800 liquid scintillation counter.

Gravitropic assay. Seeds of cress (*Lepidium sativum*) were allowed to germinate on agar (0.75%) for 24–26 hr in darkness at 20–22°. Seedlings were selected to be at about the same stage of growth and development, with the roots approximately 10 mm long. The root tips were embedded in 0.75% agar containing the substance under test at the required concentration of the substance under test in a Petri dish. A line was drawn on the under side of the dish and the tips were placed against the leading edge of this line. Four seedlings were placed in each dish and there were two replicates per concentration. The dishes were then placed vertically with the tips pointing downwards for 1 hr. They were then turned so that the roots were horizontal and grown in darkness for 18–20 hr. Roots which reacted to gravity bent downwards, while those where the response was affected either turned to a lesser degree or continued to grow in the direction they were originally pointing. The lowest concentration of the test substance at which the substance completely abolished the ability to react to gravity was recorded.

Chemicals. The chemicals used were synthesized by standard methods [3, 14] or obtained from the Aldrich Chemical Company.

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