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AUXINS II: THE EFFECT OF CHLORINATED INDOLYLACETIC ACIDS ON PEA STEMS

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Key Word Index—*Pisum sativum*; Leguminosae; pea; auxins; receptor site; structure-activity correlation.

Abstract—A series of chlorinated indolylacetic acids was assessed for auxin activity on pea stem sections. It is suggested that the activities shown are reasonably consistent with a receptor site theory of structure–activity previously proposed [1].

INTRODUCTION

During the last few years the deficiencies of earlier auxin structure–activity correlations [2] have been recognized [3] and several new theories have been proposed [1, 4–6]. In the theory put forward by one of us [1], an auxin receptor site was postulated. The concept of auxin receptors now appears to be firmly established [7]. Several specific auxin-binding sites have been isolated and can be regarded as receptor candidates [8–14]. Affinity labels have also been used with some success to investigate the binding sites [13]. It would therefore seem that auxin receptors may in fact exist, so that further development of auxin structure–activity correlations in terms of possible receptors is justified. The synthesis of a systematic series of mono- and several previously unavailable dichloro-substituted indolylacetic acids [15] renders further development possible because they are closely related to the natural hormone, and are likely to achieve their effects by a common mode of action.

The site postulated is a composite one derived from auxin activities observed in a range of species and a variety of assays. It is nevertheless possible that there may be receptor site differences between species, and that derived for any one species may differ in detail from the composite model. The effect of the chloroindolylacetic acids on pea stems was therefore examined in an attempt to define further the auxin receptor site.

RESULTS AND DISCUSSION

Activities of the indolylacetic acids are analysed by reference to a map of the auxin receptor site which has previously been proposed [1]. The map is conceived as being complementary to the IAA molecule, and is shown in Fig. 1. It was proposed that the area which accepts the indole ring was planar and electrophilic in nature, extending beyond the boundaries of the indole ring. This area is divided into areas designated Ar_1 and Ar_2, with the surrounding areas marked a–f, and is called the electron acceptor. The area corresponding to the methylene carbon of IAA is

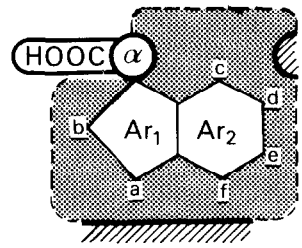


Fig. 1. The auxin receptor site.

also shown (the α area) together with the carboxyl acceptor. The hatched areas correspond to areas of steric obstruction. With the compounds tested here, the steric requirements of the carboxyl acceptor and the α area have been met, so that differences in activity may be due to differences in ability to bind to the electron acceptor. Binding ability will be affected by steric considerations, the ability of the chlorine atoms to bind to the areas surrounding the Ar_1–Ar_2 area, and by electronic effects of substituents on the indole ring, which would affect the ability of that ring to bind to the Ar_1–Ar_2 area. Electronic effects on the acidity of the carboxyl group would not be expected to be a dominant factor, firstly, because such effects would be minimized by the buffering effect of the intervening methylene group, and secondly, because a variety of acetic and benzoic acids which vary widely in their chemistry and hence their electronic effects on the acidic group are known to have high auxin activity. In particular, there are benzoic acid auxins known where electron donating and withdrawing substituents directly affect the carboxylic acid [1].

The IAA molecule is represented in Fig. 2 and the way in which monochloro derivatives would overlay the site is represented in Fig. 3. The various dichloro derivatives would be represented by appropriate composites of these diagrams.

Dose–response curves for the monochloro derivatives are shown in Fig. 4 and the dichloro derivatives

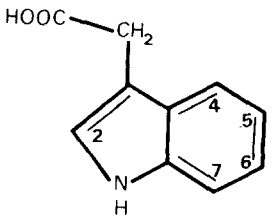


Fig. 2. Indol-3-yl acetic acid.

in Fig. 5. It would be expected that the natural hormone (i.e. IAA itself) would be an agonist [16] with respect to the receptor sites which are involved in the elongation response.

It can also be noted that 4-chloroindolylacetic acid and related chloro compounds are natural substances in *Pisum*, as well as *Vicia faba* and *Lathyrus* sp. [17–22], so that 4-chloro IAA would also be an agonist. Regarding these natural compounds as being able to elicit a full response, it can be seen from Fig. 4 that the other monochloro compounds can also give rise to a full response and are thus auxin agonists. If they have approximately equal efficacy, i.e. capacity to initiate a response [23]—then the activity of these compounds is a measure of their capacity to bind to the site, which has been termed affinity [23]. The concepts of affinity and efficacy appear to be generally accepted in animal pharmacology [24], so that their application here would seem reasonable.

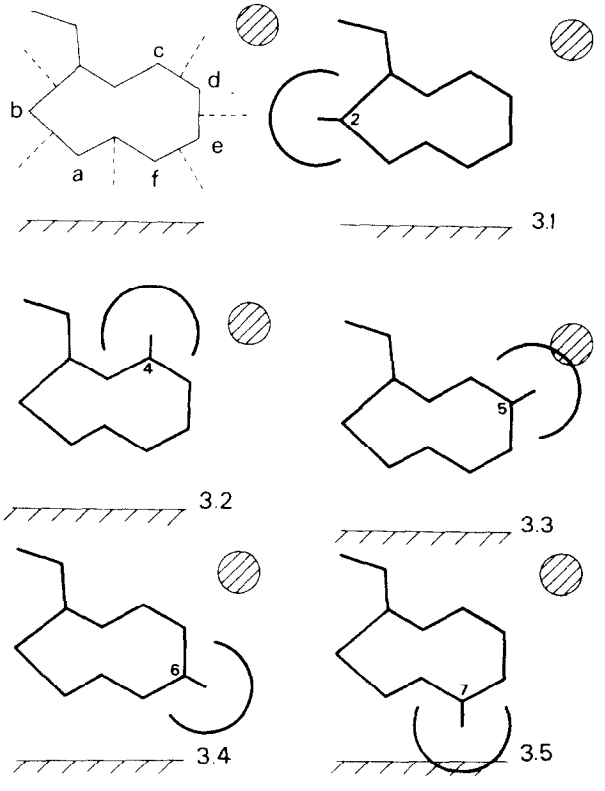
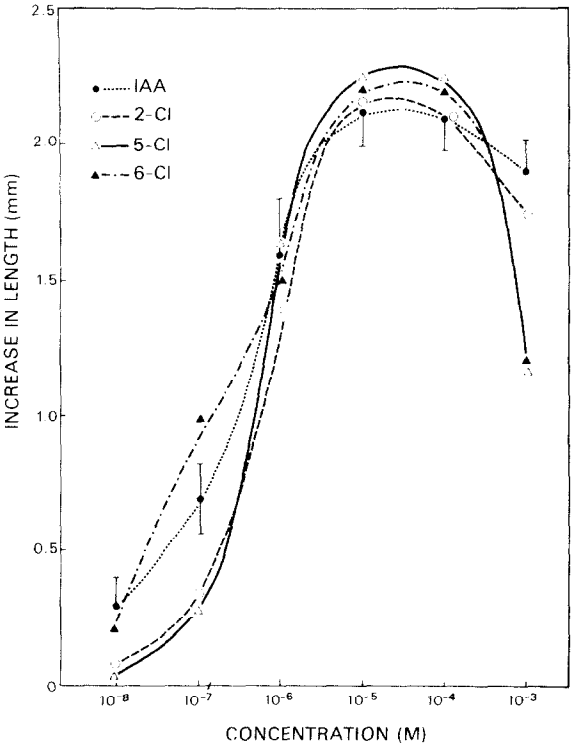


Fig. 3. Overlap of monochloroindolylacetic acids with the receptor site.



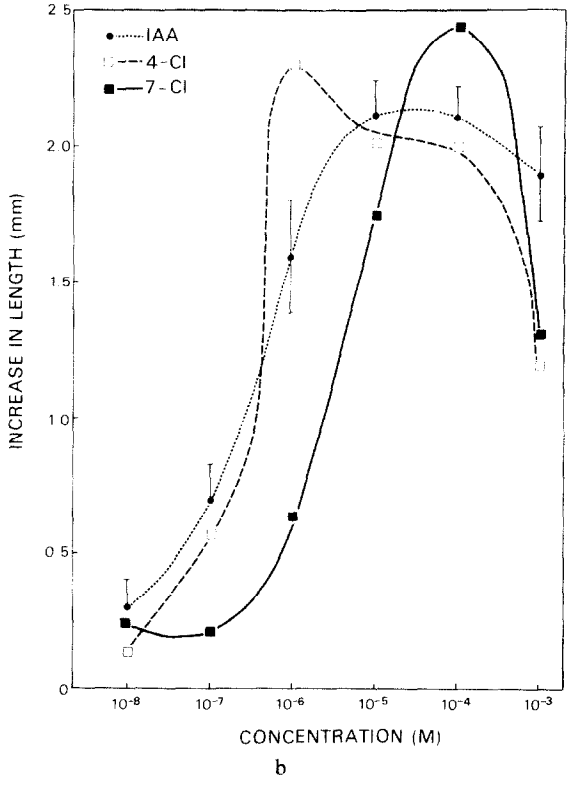


Fig. 4. Dose–response curves of monochloroindolylacetic acids on pea stem sections. ● = IAA. A. 0 = 2-, △ = 5- and ▲ = 6-chloro. B. □ = 4-, ■ = 7-chloro. Bars represent twice the s.e. for IAA for a single experiment s.e. were of similar magnitude for the other compounds.

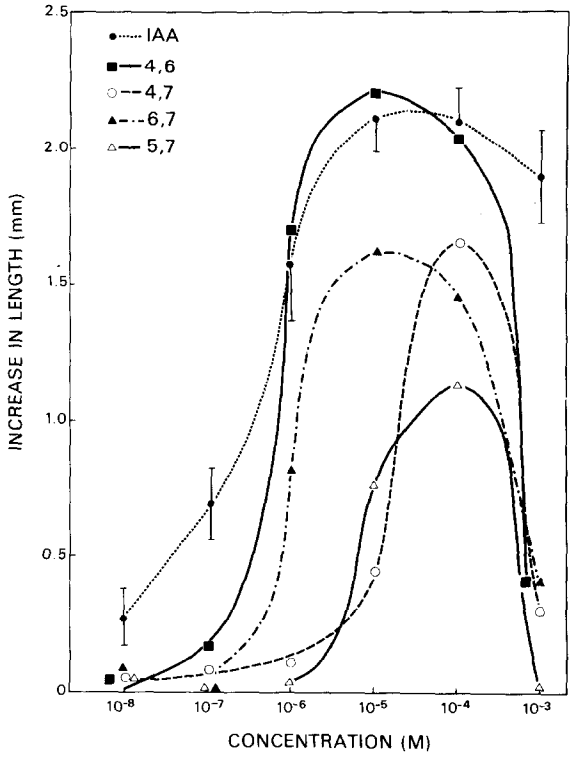


Fig. 5. Dose–response curves for dichloroindolylacetic acids on pea stem sections. ● = IAA, ■ = 4,6-dichloro, △ = 5,7-dichloro, ○ = 4,7-dichloro, ▲ = 6,7-dichloro.

Dose–response curves can be used to compare relative affinities of ligands with the receptor. The further the dose–response curve is to the right, the lower the ligand affinity [25]. Also, where a given ligand can give the same maximal response as another ligand, it is indicative that there is a closely similar interaction with the recognition site [25]. It can be seen from Fig. 4(A) that the dose–response curves for the 2-, 5-, and 6-chloro derivatives do not differ greatly from that of IAA. Higher activity than measured for the 2-chloro compound is possible, however, because this compound is unstable [26]. Substantial and reproducible differences were found with both the 4- and 7-chloro derivatives (Fig. 4B). The former compound would appear to have a higher affinity than IAA, achieving its maximum effect at a 10-fold lower concentration, while the latter appears to have a 10-fold lower affinity.

Comparison of these results with those of other workers is rendered difficult by the fact that generally only one value for overall activity is reported although the high activity for 4-chloro IAA is consistent with its activity on *Avena* [27]. The difficulty of assessing relative activity has previously been alluded to by Bottger *et al.* [27] and it can be seen here that quite different values for relative activity could be obtained depending upon which point of the curve is chosen as the point for assessment. Our conclusions appear to be at variance with those of Porter and Thimann [26] using the split pea stem assay; they found all monochloro compounds to be more active than IAA. However, their conclusion was based on a single value type of assessment. It is suggested that valid comparisons can best be made only when full dose–response curves are available.

The lower affinity for the 7-substituted compound is consistent with the postulated steric obstruction in the f area as shown in Fig. 3.5, while the higher affinity of the 4-chloro compound may indicate strong binding to the c area. This would not be inconsistent with the activities of the benzoic acids which are considered to overlay and bind to this area [1]. The area of weak steric obstruction between the c and d areas previously postulated [1] is not observable in the 5-chloro compound.

With respect to the dichloro compounds, only the 4,6-dichloro compound, which overlies the unobstructed c and e areas has activity as high as IAA (Fig. 5). The overlaying of the site by this molecule is represented by a composite of Figs. 3.2 and 3.4. The remaining compounds did not elicit a response as great as that of IAA, so that equal efficacy cannot be concluded. The 6,7-compound achieves its maximum effect at 10^{-5} M, which is the same as that for IAA. The 5,7-compound which overlies both the postulated obstructed areas gives a response which is only about half that of IAA, and achieves it only at a 10-fold higher concentration. The 4,7-analogue can give rise to 80–90% of the full response, but again only at the higher concentration. It is suggested that the generally lower activity of the 7-substituted compounds and the even lower activity of the 5,7-dichloro compound is consistent with the receptor site map previously proposed, although the electronic effects of substituents cannot be excluded here.

The discussion of biological activities in terms of receptor sites is open to objection, but it is suggested that it is justified at least as a guide to further research if the inherent assumptions are recognized.

In the present case, conclusions with respect to the receptor site must be qualified by assumptions with respect to mode of action, uptake, mobility, and removal of the hormone by such means as degradation, conjugation or sequestration. The assumption of a common mode of action would seem justified because of the close chemical similarity of the compounds which differ only in the position and number of unreactive chlorine atoms (except for the 2-chloro analogue where the halogen is reactive). Uptake can be affected by non-specific factors such as lipophilicity. This factor was assessed from partition data obtained in the reference system *n*-octanol–water. As can be seen from Table 1, the partition coefficients fall into two main groups, depending on whether the molecules possess one chlorine atom or two, with the dihalogenated compounds being more lipophilic. The unchlorinated parent compound is more hydrophilic than the two groups. This result is quite different from the observed structure–activity pattern, so that it is concluded that non-specific lipophilicity is not a dominant factor. Specific hormonal uptake and transport are considered to be controlled by pharmacological receptors [24] so that the results observed would reflect activity with respect to these receptors as well as those which directly give rise to cell expansion. Termination of agonist action, however, relies on non-receptor tissue components.

Table 1. Partition coefficients of indolylacetic acid and chlorinated indolylacetic acids

Compound	P*	Compound	P
IAA	0.11	4,6-dichloro IAA	3.72
4-chloro-	0.48	4,7-dichloro	2.44
5-chloro-	0.56	5,7-dichloro	3.92
6-chloro-	0.88	6,7-dichloro	3.02
7-chloro-	0.36		

*$P = C_{octanol}/C_{buffer}$ measured at 21° where C is the molar concentration.

The natural hormones being potent agonists should have higher affinity for their receptors than they have towards metabolism or sequestration molecules [24]. This is not necessarily the case for synthetic compounds, unless they are shown to be potent agonists. However, the fact that widely differing chemical types can give rise to results consistent with the receptor site map [1] would indicate that differences in chemical breakdown rate that are not controlled by receptors may not be an important factor in these assays.

With the above qualifications, it is concluded that there is evidence to suggest that the receptors involved in pea stem elongation possess features which are consistent with the receptor map previously proposed, and that there may be efficacy and affinity differences between the closely related molecules tested. They therefore may be suitable models for further receptor site investigations.

EXPERIMENTAL

Chemicals. IAA was obtained from the Aldrich Chemical Co. 2-Chloro IAA was made by the method of ref. [26]. The gift of the other chlorinated indolylacetic acids [15] by Dr. K. C. Engvild is gratefully acknowledged.

Measurement of partition coefficients. These were measured in *n*-octanol–Pi buffer (pH 7; 1:1) using UV spectroscopy to measure concns in both phases. Values quoted are averages of at least two separate measurements.

Auxin activity was assessed in accordance with the methods outlined in the *Agricultural Handbook* of the United States Department of Agriculture (1968). Seeds of *Pisum sativum* cv Victory Freezer were soaked in running H_2O for 7 hr and planted in a mixture of Perlite and Vermiculite (1:1). Plants were grown in total darkness at 26°. All observations and manipulations were made under green safety light. Plants received 20 min of red light (Philips red fluorescent tube TL 40W/15, 263 W/cm² 600–800 nm of which 10% was between 700 and 800 nm), before harvesting. When the fourth internode was not more than 2 mm long, 6-mm segments were cut from the apical end of the third internode. After excision, at least 10 stem segments were placed in each Petri dish containing 15 ml of the test soln. The segments were allowed to elongate for 24 hr in the dark at 26° and were subsequently measured to the nearest mm.

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