New Auxin Analogs. Possible Probes for Auxin Receptors

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Based on structure-activity relationship studies, auxin analogs that can be covalently bound to a polymeric support are proposed. Molecular modeling studies were carried out by comparing different parameters of substituted phenoxyacetic acids with their auxin activity. A good correlation of the activity with the size and shape of the HOMO orbital of the acids was found. Accordingly, analogs with a substituent in the 5 position of the aromatic ring, capable to be bound to a polymeric matrix were synthesized and their auxin activity was evaluated with the wheat coleoptile elongation test. Compounds with a hydroxymethyl- and with a carboxymethoxy- substituent were active in this test. Their use as probes for the 2,4-D receptor is proposed.

Introduction

Theories on auxin structure-activity correlations have been widely reviewed, (Muir and Hansch, 1955; Katekar, 1979; Edgerton et al., 1994). Hypothetical receptor sites on specific cellular recognition proteins are proposed, which bind the hormone and initiate a sequence of events (signal transduction), that culminate in a characteristic physiological or biochemical response.

Three approaches have been used for auxin structure-activity correlation studies: the activity measurement of adequate series of compounds and estimation of the molecular characteristics which determine their activity; binding studies with suitable compounds on isolated receptors and lastly, molecular modeling studies.

Studies by Porter and Thimann (1965), Wain and Fawcett (1969), Kaethner (1977), Lehmann (1978) and Katekar and Geissler (1983), resulted in models of auxin binding sites and had the long-term purpose of knowing the chemical nature of receptors, which might assist in their isolation and characterization.

On the other hand, a large number of auxin binding proteins located in more than one type of cellular membranes have been reported (Jones et al., 1998; Libbenga et al., 1986). They are characterized as ABP’s (auxin binding proteins) by their susceptibility to covalent photolabeling by tritiated azido-indole-3-acetic acid (Venis and Napier, 1985). The multiplicity of auxin binding sites opened the problem of auxin site modeling for re-investigation.

ABP1 is one of the best-studied binding proteins. Ray (1977), measured dissociation constants of 45 auxin analogs with ABP1, his data on auxin binding represent the only intensive characterization of a single auxin-binding site analyzed in vitro published to date. With these data, Edgerton et al. (1994) modeled the auxin-binding site of the maize’ ABP1. These researchers proposed an auxin phytophore that incorporates the key structural features of the three compounds that bind the protein with the highest affinity: NAA (naphthalic acid), N2AA (2naphthalic acid) and IAA (indoleacetic acid). The ABP1 binding site according with these authors, consists of a platform to which the indole or naphthalene rings bind and a somewhat flexible region, which interacts with the carboxylic acid group.

Abbreviations: 2,4-D, 2,4-dichlorophenoxyacetic acid; ABP, auxin binding protein; ABP1, auxin binding protein 1 from maize; CNDO, complete neglect of differential overlapping; HOMO, highest occupied molecular orbital; IAA, indoleacetic acid; INDO, intermediate neglect of differential overlapping; Kd, dissociation constant; LogP, logarithm of the partition coefficient; LUMO, lowest unoccupied molecular orbital; N2AA, 2-Naphthalic acid; NAA, Naphthal acetic acid; PMR, Proton magnetic resonance; s, strong; vs, very strong.

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A clear correlation between the biological activity and the auxin affinity constants is apparent in Ray’s work (1977), however, one of the highest discrepancies of the model is the low affinity of 2,4-D which has a large auxin activity and does not have a significant binding. Several other compounds among those assayed by Ray (1977) clearly demonstrate the pitfalls of bioassay based measurements in constructing receptor models.

Molecular modeling techniques have proven useful in aiding the identification and design of bioactive compounds. The use of semi-empirical methods for calculating the frontier orbitals of IAA and aryl substituted phenoxyacetic acids in relation with auxin activity, tended to implicate the LUMO (lowest unoccupied molecular orbital) at the ortho position of the aromatic ring in the mechanism of binding (Block and Clements, 1975). Block et al. (1977) carried out calculations with the INDO method (intermediate neglect of differential overlap) on a series of 5-substituted indole-3-acetic acid derivatives and found that the Mulliken bond order calculations of the N-H bond in these compounds did not reflect their auxin activity. The results did not correspond to observed N-H stretching frequency changes either. The ground state triplet oxygen would be capable of addition across the 2–3 bond of the indole in a ring opening reaction, but the calculated HOMO orbital was almost the same magnitude in all the auxins.

Russian researchers determined the electron configuration and molecular potential of IAA using CNDO-2 and Molpot-2, respectively, and found that the carboxylic C and O had the largest positive and negative charges respectively, and that the molecule had three local extreme of molecular potential. These data were discussed in relation to auxin activity and interaction with receptors (Ramishvili et al., 1990).

Cohen and Bandursky (1982) had shown that auxin conjugates are storage forms that are hydrolyzed to the free hormone as required. Duddeck et al. (1989) and Kojic-Prodic et al. (1991) studied the conformation of the lateral chain of IAA conjugates in an attempt to explain the interaction of free and bound auxin in some plant systems. They found that the IAA’s lateral chain does not change significantly in the conjugates, although their lipophylicity varies considerably and might explain their poor physiological activity.

In this work, with the recent development of computer aided molecular modeling and considering the controversial topic of structure-activity related to auxin receptors, a general study was carried out of the properties which could be relevant for auxin activity. The objective of this study was to propose new analogs of 2,4-D that could be covalently bound to a polymeric support in order to permit the isolation of the 2,4-D-receptor, which according to Ray’s data (1977) might be different to the IAA receptor. The analogs were synthesized and their biological activity was measured.

Materials and Methods

Molecular modeling

All calculations were performed using the Hyperchem program (Hyperchem release 5.01, 1999. Hypercube. Inc. 419 1115 NW 4th Street, Gainesville FL 32601, USA. Phone (352) 371–7744, Fax (352) 371–3662). Molecular mechanics (MM+) and semiempirical methods (AM1, PM3) were used as supplied by Hypercube. Calculations were performed initially with MM+ and then with AM1 or PM3. All geometries were completely optimized to 0.0001 convergence.

Chemical methods

2,4-Dichlororesorcinol (I): Prepared according to Beilstein (1943) from resorcinol (0.1 mol) and sulfuryl chloride (0.2 mol). The product was purified by sublimation. Yield 60%. m.p. 105 °C. IR (infrared), (KBr) vs (very strong) (1476, C-H), s (strong) (3520, O-H; 1148, C-O; 724, C-Cl), m (medium) (1586, C=C) cm⁻¹. PMR (proton magnetic resonance) (90 MHz, DCCl₃) δ 5.58 (s, 2, OH), 6.72 (s, 1, CH), 7.28 (s, 1, CH).

2,4-Dichloro-5-hydroxyphenoxyacetic acid (II): 2,4-Dichlororesorcinol (1.033 g, 5.8 mmol) in anhydrous ethanol was made to react with 0.3 g (5.4 mmol) of potassium hydroxide in 10 ml of anhydrous ethanol. 0.708 g (7.93 mmol) of chloroacetic acid in anhydrous ethanol was added during one hour. After refluxing 30 hours, the solvent was evaporated; the residual crystalline solid was dissolved in 5% NaOH solution and extracted with ethyl acetate. After acidification of the aqueous phase, 2,4-dichloro-5-hydroxyphenoxyacetic acid
was filtered and recrystallized from dichloromethane-hexane. Yield 30%. m.p. 161–162 °C. IR (KBr) vs (1754, C=O; 1198, C-O), s (3406–3120, O-H), m (740, C-Cl) cm⁻¹. PMR (90 MHz, DCCl₃) δ 4.63 (s, 2, CH₂), 5.62 (s, 1, OH), 6.55 (s, 1, CH), 7.28 (s, 1, CH), 10.5 (s, 1, COOH).

5-Acetoxy-2,4-dichlorophenoxyacetic acid (III): 70 mg (0.3 mmol) of (II) was acetylated with acetic anhydride / NaOH according to Vogel (1978) and recrystallized from methanol. Yield 83%. m.p. 133–135 °C. IR (KBr) vs (1756, C=O; 1192, C-O), s (3404, O-H; 724, C-Cl) cm⁻¹. PMR (90 MHz, DCCl₃) δ 2.2 (s, 3, CH₃), 3.4 (s, 3, CH₂), 4.2 (s, 2, CH₂), 6.5 (s, 1, CH), 7.4 (s, 1, CH). (VI):

2,4-Dichloro-5-(methoxycarboxy­methoxy)-phenoxyacetic acid (IV): 4 g (100 mmol) of NaOH were made to react with 2 g (11.2 mmol) of (I) in 50 ml anhydrous ethanol until dissolution, followed by 6.3 g (67 mmol) of chloroacetic acid in anhydrous ethanol. After refluxing 50 hours, the solvent was evaporated, the residue acidified (pH 2) and the solution extracted with ethyl acetate. The organic extract was evaporated and the product was recrystallized from ethyl acetate. Yield 79%. m.p. 227–228 °C. IR (KBr) vs (1734, C=O; 1192, C-O-C), s (3404, O-H; 724, C-Cl) cm⁻¹. PMR (90 MHz, DCCl₃) δ 4.7 (s, 2, CH₂), 6.8 (s, 1, CH), 7.38 (s, 1, CH), 7.5 (s, 1, CH), 11.8 (s, 1, CHO).

Methyl-(5-carboxymethoxy)-2,4-dichlorophenoxyacetic acid (V): 5-(Carboxymethoxy)-2,4-dichlorophenoxyacetic acid (0.100 g, 0.34 mmol) was made to react with diazomethane in ether. The dimethyl ester was recovered as a white, crystalline solid. Yield 98%. m.p. 138–139 °C. IR (KBr) vs (1732, C=O), s (1268, C-O-C; 748, C-Cl) cm⁻¹. PMR (90 MHz, DCCl₃) δ 3.8 (s, 3, CH₃), 3.85 (s, 2, CH₂), 4.75 (s, 2, CH₂), 6.6 (s, 1, CH), 7.4 (s, 1, CH), 9.5 (s, 1, COOH).

2,4-Dichloro-5-(methoxycarboxy­methoxy)-2,4-dichlorophenoxyacetic acid (VI): 50 mg (0.18 mmol) of (III) was made to react with diazomethane in ether. The pure white solid was recovered. Yield 80%. m.p. 136–138 °C. IR (KBr) vs (1612, C=C; 1210, C-O-C), s (1762, C=O), m (2932, C-H; 772, C-Cl) cm⁻¹. PMR (90 MHz, DCCl₃ + MeOD) δ 2.24 (s, 3, CH₃), 3.4 (s, 3, CH₂), 4.2 (s, 2, CH₂), 6.5 (s, 1, CH), 7.4 (s, 1, CH).

Methyl-(5-acetoxy-2,4-dichlorophenoxy) acetate (VII): Esterification of 5-carboxy­methoxy-2,4-dichlorophenoxyacetic acid (2.4 mmol) with one equivalent of methyl alcohol was carried out using p-toluene sulfonic acid as catalyst. Yield 15%. m.p. 133–135 °C. IR (KBr) vs (1756, C=O; 1218, 1182, C-O-C), m (3358–2956, O-H), w (772, C-Cl) cm⁻¹. PMR (90 MHz, DCCl₃ + MeOD) δ 3.6 (s, 3, CH₃), 3.85 (s, 2, CH₂), 4.75 (s, 2, CH₂), 6.6 (s, 1, CH), 7.45 (s, 1, CH). 2,4-Dichloro-5-methoxybenzaldehyde (VIII): It was prepared from m-anisaldehyde (0.1 mol) and sulfuryl chloride, (0.2 mol). Yield 13%. m.p. 98–100 °C. IR (KBr) vs (1704, C=O), s (1210, C-O), m (648, C-Cl) cm⁻¹. PMR (90 MHz, DCCl₃) δ 3.9 (s, 3, OCH₃), 7.1 (dd, 2, J = 3 and 6 Hz, CH), 10.1 (s, 1, CHO).

2,4-Dichloro-5-hydroxybenzyl alcohol (X): 2,4-Dichloro-5-methoxybenzaldehyde (VIII) was hydrolyzed with hydrobromic acid 48% and acetic acid (v:v). The reaction mixture in a Teflon container was placed in a stainless-steel reactor at 140 °C during 48 hours. The solid product was recrystallized from ethylacetate-hexane. Yield 76%. m.p. 133–135 °C. IR (KBr) vs (1680, C=O), s (3238, O-H), m (688, C-Cl) cm⁻¹. PMR (90 MHz, DCCl₃ + DMSO) δ 7.1 (s, 1, CH), 7.5 (s, 1, CH), 7.7 (s, 1, OH), 10.5 (s, 1, CHO).

2,4-Dichloro-5-hydroxybenzaldehyde (IX): 2,4-dichloro-5-methoxybenzaldehyde (VIII) was reduced with excess sodium borohydride in anhydrous ethyl alcohol as solvent, at 0 °C during 2 hours. Yield 66%. m.p. 102–104 °C. IR (KBr) s (3212, O-H), m (1188, C-O), w (688, C-Cl) cm⁻¹. PMR (90 MHz, DCCl₃ + DMSO) δ 4.8 (s, 2, CH₂), 4.9 (s, 1, OH), 6.9 (dd, 2, J = 3 and 4 Hz, CH), 10 (s, 1, OH). 2,4-Dichloro-5-(hydroxymethyl)-phenoxyacetic acid (XI): A wet solid mixture of sodium phenolate and sodium chloroacetate was obtained by neutralization of a mixture of phenol (X) (50 mmol) and chloroacetic acid (50 mmol) with a solution of sodium hydroxide (50%) (50 mmol) and evaporation of water in vacuum. The mixture was irradiated 16 minutes in a commercial microwave oven (350–420W), (Villemin and Hammadi, 1996). The mixture was redissolved in 2 ml of water, acidified to pH 3 with hydrochloric acid and collected by filtration as a white solid. Yield 60%. m.p. 162–164 °C. IR (KBr) vs (1738, C=O), s (3358, O-H; 1204, C-O-C), m (686, C-Cl) cm⁻¹. PMR (90 MHz, DCCl₃ + DMSO) δ 3.3 (s, 2, CH₂), 3.8 (s, 1, OH), 4.8 (s, 2, OCH₂CO), 7.1 (dd, 2, J = 3 and 8 Hz, CH), 9.5 (s, 1, COOH).
5-Carboxymethoxy-2,4-dichlorobenzyl acetate (XII): A solution of BF$_3$ Et$_2$O (1.7 µl; 0.01 mmol) in Ac$_2$O (0.13 ml) was added dropwise to a suspension of XI (17 mg; 0.07 mmol) in THF (2 ml) with stirring under ice cooling. After stirring 1 h under the same conditions, the reaction mixture was evaporated to dryness, neutralized to pH 7 with sodium bicarbonate solution and extracted with chloroform. The residual brown crude product was recrystallized from chloroform-cyclohexane. Yield 86%. m.p. 139–140 °C. IR (KBr) vs (1740, C=O), s (3350, O-H; 1236, C-O-C), m (696, C-Cl) cm$^{-1}$. PMR (90 MHz, CDCl$_3$) $6$ 2.05 (s, 3, CH$_3$), 4.35 (s, 2, CH$_2$), 4.7 (s, 2, CH$_2$), 6.9 (s, 1, CH), 7.3 (s, 1, CH), 9.0 (s, 1, COOH).

Biological methods

Seeds of wheat (Triticum aestivum, variety Galvez M-87) from CEVAMEX (Campo Experimental del Valle de México), INIFAP, were used in this work. Seeds were grown in moist cotton during 70 hours at 24 °C in darkness. After 50 hours of imbibition, the seedlings were selected for size. Each experiment was carried out at room temperature and with minimum light.

Auxinic activity measurement. Wheat coleoptile elongation test

Excised sections of 6 mm long of coleoptiles 2 cm long were cut with a sharp blade under red illumination (10 watts) eliminating the top 3 mm of the plantule. A capillar glass tube 0.7 mm diameter was introduced into the coleoptile segment to eliminate the primordial leaf and to maintain it in a vertical position. Ten coleoptile segments were made to float in 15 ml of solution of each of the synthesized compounds at stated concentration, using Petri dishes of 9 cm of diameter. Experiments were carried out using distilled water and 2,4-D as controls. After 24 hr. at 25 °C in darkness, the length of the sections was measured and the results were expressed as a percentage with reference to the length of the water controls. Results in Table I show data of duplicate experiments carried out with all the synthesized compounds at the same time.

Results and Discussion

The objective of this work was to synthesize and test 2,4-D analogs that could be covalently bound to a polymeric support. This binding has to be done either through the carboxyl group, the methylene of the side chain or through one of the three free positions of the aromatic ring. Since the importance of the free carboxyl group for the auxin activity is well documented, (Wain and Fawcett, 1969) and because we had coupled 2,4-D to a Sepharose column by forming an amide with the auxin-carboxyl group and lysine (Perez Flores et al., 1984) and showed that the protein which bind the column is heterogeneous, we eliminated this position for the coupling. Works of other authors, (Kaethner, 1977; Lehmann, 1978; Katekar, 1983), support the importance of the absence of steric hindrance in the side chain for the maintenance of the activity, therefore, the binding through the lateral chain methylene was also discarded. Binding through the 5 position of the aromatic ring was proposed as the best option for the purpose (substitution at position 6 according to Wilkins (1969) suppresses the auxin activity and substitution in position 3 reduces this activity) (Smith and Mohsin, 1970).

<table>
<thead>
<tr>
<th>Compound No.</th>
<th>10$^{-3}$</th>
<th>10$^{-4}$</th>
<th>10$^{-5}$</th>
<th>10$^{-6}$</th>
<th>10$^{-7}$</th>
<th>10$^{-8}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>III</td>
<td>82.3±6.0</td>
<td>96.7±2.0</td>
<td>99.4±2.0</td>
<td>96.5±0.6</td>
<td>92.4±1.0</td>
<td>83.0</td>
</tr>
<tr>
<td>IV</td>
<td>70.2</td>
<td>93.6</td>
<td>94.7±1.2</td>
<td>95.9±2.3</td>
<td>93.6</td>
<td>81.9±5.8</td>
</tr>
<tr>
<td>V</td>
<td>92.1±1.5</td>
<td>102.9±2.3</td>
<td>101.7±1.2</td>
<td>97.1±1.1</td>
<td>107.5±1.0</td>
<td>94.1±2.9</td>
</tr>
<tr>
<td>VI</td>
<td>118.7±2.9</td>
<td>120.5±2.3</td>
<td>109.9±2.3</td>
<td>113.5±5.5</td>
<td>109.9±3.5</td>
<td>97.1±3.5</td>
</tr>
<tr>
<td>VII</td>
<td>97.4±3.9</td>
<td>127.5±1.2</td>
<td>118.1±3.0</td>
<td>104.1±3.4</td>
<td>102.3±1.7</td>
<td>93.7±2.4</td>
</tr>
<tr>
<td>XI</td>
<td>72.5</td>
<td>127.4</td>
<td>104.1±3.4</td>
<td>102.3±1.7</td>
<td>97.0±1.1</td>
<td>95.9±1.1</td>
</tr>
<tr>
<td>XII</td>
<td>70.2</td>
<td>94.7±1.0</td>
<td>103.5±1.7</td>
<td>104.1±1.2</td>
<td>93.7±2.4</td>
<td></td>
</tr>
<tr>
<td>2.4-D</td>
<td>79.7±1.1</td>
<td>138.3±0.7</td>
<td>124.7±1.0</td>
<td>108.1±0.5</td>
<td>97.0±1.1</td>
<td>95.9±1.1</td>
</tr>
</tbody>
</table>
Before starting the synthesis of presumably active branched analogs of 2,4-D to bind a polymeric support, theoretical molecular modeling studies were performed using the Hyperchem program. A variety of phenoxyacetic acid analogs with different positions and type of substituent were used for the study. Different parameters were considered for their comparison with auxin activity such as log P, contact area, molecular volume, molecular refractivity, mass, atomic energy, heat of formation, distances between different atoms in the molecules, angles of the side chain, energy of the HOMO orbital, and energy of the LUMO orbital. To perform the study, the conformation of all the compounds was optimized at a convergence limit of 0.0001 with an ab initio method. An exhaustive search of the global minimum was performed also by rotating the Ar-O-C angle and the O-C-C=O torsion angle of the side chain. A good correlation of the activity with the size and shape of the HOMO orbital was found: the inactive compounds have a translapping orbital from the ring or the ortho substituent with the orbital of the ether, and the active compounds did not overlap. Fig. 1 shows the HOMO orbitals of 2,4-dichlorophenoxyacetic acid and of 2,3-dichlorophenoxyacetic acid an inactive acid. It can be seen that the inactive acid has an overlapping HOMO orbital in the lateral chain, whereas the auxin 2,4-D has a separate lateral chain orbital, which is indicated by an arrow.

Two of the target compounds: 2,4-dichloro-5-carboxymethoxy-phenoxyacetic acid (IV, Fig. 2) and 2,4-dichloro-5-hydroxymethyl-phenoxyacetic acid (XI, Fig. 3) are also shown in Fig. 1. They had a similar shape and density of the HOMO as 2,4,5-trichlorophenoxyacetic acid, which are active auxins. These comparisons led us to predict that these compounds might have auxin activity. With this information we proceeded to...
synthesize and test the auxin activity of these compounds.

The synthesis of the proposed compounds was carried out using resorcinol or anisaldehyde as starting materials.

The route for the synthesis of 5-alkyloxy compounds starting from resorcinol is shown in Fig 2. Chlorination of resorcinol produced 2,4-dichloro resorcinol (I), which was mono or dialkylated with chloroacetic acid to produce compounds II or IV which were identified by their expected IR and PMR spectra (see chemical methods).

5-Hydroxymethyl derivatives of 2,4-D were obtained from anisaldehyde through the synthetic route shown in Fig. 3. m-Anisaldehyde was chlorinated using sulfuryl chloride as in the case of resorcinol. Although the yields were poor, the isolation and purification of 2,4-dichloro-5-methoxybenzaldehyde (VIII), showed the expected physical and spectroscopic constants. The hydrolysis of the methoxy group and the reduction of the aldehyde group produced the alcohol X which was made to react with chloroacetic acid by the method of Vilelemin and Hammadi (1996) using a microwave oven to produce the target compound (XI).

The interaction with a receptor is not the only factor that determines the biological activity; partition coefficients are also very important. A modification of the solubilities of some compounds was carried out by making the alcohol or phenol ace-

Fig. 3. Synthetic pathway for 5-carboxymethoxy- derivatives of 2,4-D. The auxin activity of compounds marked with an asterisk was tested.

the acid proton is able to form a hydrogen bond with the acetate group oxygen which restricts the free rotation of the acid chain; this might be the reason of its inactivity.

In conclusion we suggest that compound IV might be bound to a polymeric support through an esterification; this reaction would produce an ester similar to VII, which might be useful for the isolation of the 2,4-D receptor. Further work is being carried out in this area.


