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Auxin Transport Inhibitors

III. CHEMICAL REQUIREMENTS OF A CLASS OF AUXIN TRANSPORT INHIBITORS

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ABSTRACT

The structural requirements of a proposed class of auxin transport inhibitors have been shown to be very similar to those required to inhibit the cress (*Lepidium sativum*) root geotropic response. A 2-carboxyphenyl group separated by a conjugated system of atoms from a second aromatic ring appears to be necessary for a molecule to have high activity.

A variety of substances which can prevent the active movement of auxin in plants is now known (17, 18, 20). It is at least possible that some of them may act by a common mechanism, and those that do would therefore be expected to have some similarities with respect to physical and chemical properties, enabling them to act at the same active site. It is known that many compounds which inhibit auxin transport also have the ability to abolish the root geotropic response (13, 18) although the nature of the relationship between these two processes has yet to be defined. The structural requirements of auxin transport-inhibiting compounds are of interest because such knowledge should assist in defining the physiological processes which are involved and lead to possible mechanisms by which they act. Recently it has been shown that a class of compounds which affect the root geotropic response can be defined by a common set of chemical and physical parameters (13), and since it may be that some of the chemicals which affect both processes achieve their result by a common mechanism, the extent to which the same parameters were required for activity with respect to auxin transport was investigated.

MATERIALS AND METHODS

Chemicals. 2-(1-Pyrenoyl)benzoic acid I (8) and 2-(1-naphthoyl)benzoic acid Id (12) were prepared by the literature methods. Preparation of 1-(2'-carboxyphenyl)-3-phenylpropane-1, 3-dione IV (6) 3,3a-dihydro-2-phenyl-8H-pyrazolo-[5,1a]isoindole-8-one V; R=H (10) 2-phenyl-8H-pyrazolo-[5,1a]isoindol-8-one VI (10) 5-phenacylidine-2(5H)-furanone IVb (6), 1-(2'-carboxyphenyl)-butane-1,3-dione IVc (6), 2-phenyl-8H-pyrazolo[5-1a]isoindol-8-one VI, and 5-(2-carboxyphenyl)-3-phenylpyrazole VII (10) has been previously described. Other chemicals were obtained from the Aldrich Chemical Company.

Auxin Transport. The transport system selected consisted of bean petioles (*Phaseolus vulgaris* var. Brown Beauty). Only petioles from healthy plants which had reached the same stage of development were used, but in general plants were between 16 and 18 days old. Seedlings were grown under glasshouse conditions under fluorescent light (18-22 C, 1,500 ft-c^{-2}).

The general technique based on that described by Keitt and Baker (16) was used. This technique was used because it was designed to measure the effect of compounds on transport, rather than uptake of IAA. Twenty petiole segments 10 mm in length were cut and placed in Perspex holders with the basal end resting on receiver planchets of 1.5 ml of 1.5% agar, containing the required concentrations of chemicals under test. Donor blocks of agar gel were prepared containing 1 μg/ml of IAA-2-^{14}C (2.5 cm diameter, 1.5 ml volume, 1.5% agar, IAA activity 30 mCi/mmol). These were placed on the apical ends of the segments, which were incubated in a humid environment for 4 hr under laboratory lighting conditions. The radioactivity transported to treated blocks after this period was determined by a gas flow counter. Acropetal movement of IAA in experiments where receiver and donor blocks were reversed was 5% of basipetal movement in untreated controls, and was not significantly different from this value in treated replicates. At least three replicates per concentration were used.

RESULTS AND DISCUSSION

It is known that compounds I through V are auxin transport inhibitors (Fig. 1) (2, 7, 14, 15, 17) and it can be seen from Figure 2 that as observed by Beyer *et al.* (3) the *iso*indolone VI and the diazole VII are also potent inhibitors of the active movement of IAA. The *iso*indolone V; R=H, has also been included for comparison; it is an analogue of DPX1840' (V; R=OCH_3) which has previously been reported as having this activity (2). All of these compounds are also powerful inhibitors of the geotropic response, for which a set of structure-activity requirements has been previously postulated (13). These requirements, represented by Figure 1, 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 systems of atoms.
5. There is also a spatial requirement which can be expressed by saying that high activity will be reached when the distance between the centers of the two extreme aromatic rings is at least 7.3 Å. Substituents on the noncarboxylated ring have been found to increase activity if they assist in the achievement of this minimum molecular size, but otherwise have little effect. Selected compounds were examined to determine whether the requirements for auxin transport activity could be the same as those for the geotropic response.

In Table I the propanedione IV was chosen as the model compound, with the other compounds—IVa, missing the carboxylic acid, IVb, missing the first aryl ring—which would be

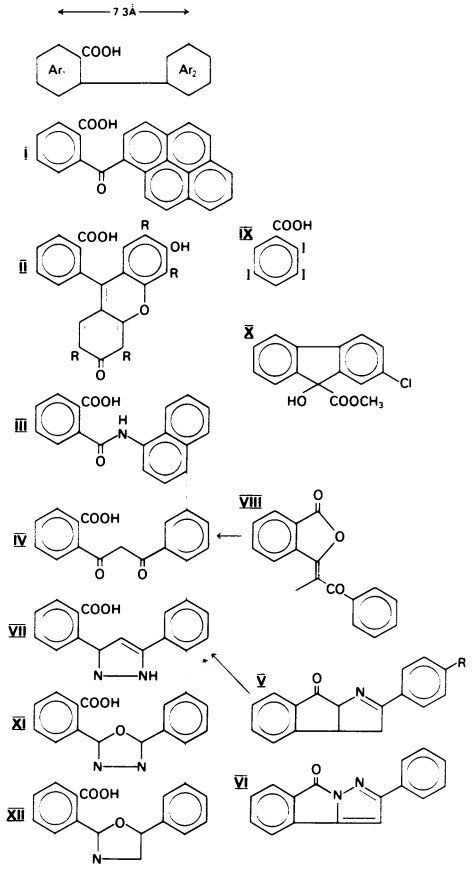


FIG. 1. Chemical structure of auxin transport inhibitors.

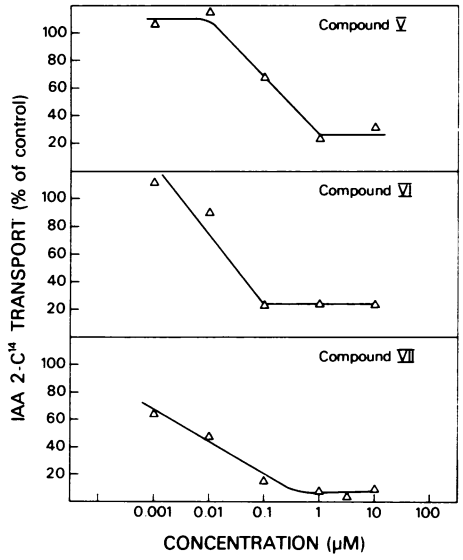


FIG. 2. Effect of compounds V, VI, and VII on IAA-2-^{14}C movement in bean petioles.

expected to open as shown (*c.f.* VIII, which is as active as its open chain form IV [7]) and IVc, missing the second aryl ring. Each compound lacking an important substituent was found to be essentially inactive, since at 100 μM they permitted transport of more than 50% of labeled IAA compared with controls. By comparison, the propanedione IV is at least 1,000 times more effective since it does not allow more than 50% through at any concentration above 0.1 μM, nor more than 8% at 10 μM. The requirement of conjugation, or perhaps planarity, is illustrated by the relative activities of fluorescein II; R=H, and phenolphthalein IIa. The latter unconjugated compound is the less active of the two (see Table II).

With respect to the length requirement, the aryl benzoic acid series was investigated. Results are shown in Table III. When the second aromatic ring is missing, as in Ia, the compound is inactive. With a second aromatic ring present and a single atom between the rings, as in Ib, the compound has some activity, and this activity is increased by a chlorine atom which has the effect of increasing the length of the molecule Ic. When the second aromatic ring is part of a naphthalene system, there is also an increase in length and activity, Id, while in I, where the extreme aromatic ring is part of a pyrene system, and the length of the molecule is thus greater than the postulated minimum length for high activity, the compound is a potent inhibitor of auxin transport. The fluoresceins also conform to this pattern. Fluorescein itself (II; R=H) which is below the minimum size, is less active than eosin (II; R=Br) (14). The activity is therefore increased by the bromine atoms, which have the effect of increasing the length of the molecule.

All of the above activities with respect to auxin transport parallel the reported activities with respect to the geotropic response (6, 9, 13, 19). We suggest, therefore, that there is a class of auxin transport inhibitors which can be defined by a set of parameters which are at least similar to the parameters deduced to be required for compounds affecting the geotropic response.

Comparison with Other Theories. It is of interest to compare these requirements with the natural and synthetic auxins. There appears to be no settled theory which will accommodate all molecules having auxin activity (1). However, many compounds having auxin activity do in fact conform to rules 1 and 2, although not to the other rules. Even here, the carboxylic acid and the aromatic ring need not be directly linked for a molecule

Table I. *Effect of Compounds Lacking an Essential Group on IAA-2-^{14}C Movement in Bean Petioles, and Comparison with Reported Root Geotropic Activity*

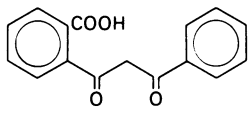
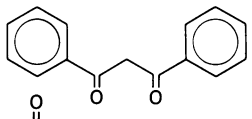
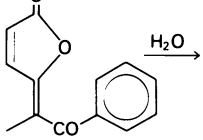
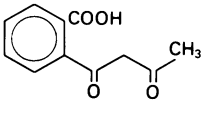
COMPOUND NO.	STRUCTURE	IAA TRANSMISSION AT 100μM		ROOT GEOTROPIC ACTIVITY (13)
		cpm	% of control	(μM)
IV		106	5	0.1
IVa		1384 ± 468	65	>100
IVb		1574 ± 229	73	>10
IVc		1258 ± 312	59	>10

Table II. *Effect of Conjugation on IAA-2-^{14}C Movement in Bean Petioles and Comparison with Reported Root Geotropic Activity*

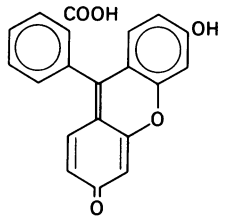
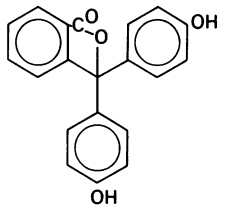
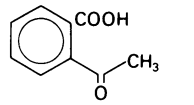
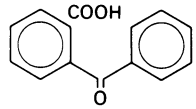
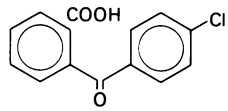
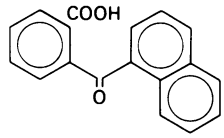
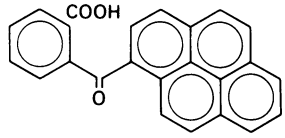
COMPOUND NO.	STRUCTURE	IAA TRANSMISSION AT 100μM		ROOT GEOTROPIC ACTIVITY
		cpm	% of control	(μM)
II		54 ± 4	6	100
IIa		768 ± 330	83	>100

Table III. *Effect of Increasing Molecular Length on IAA-2-^{14}C Movement in Bean Petioles and Comparison with Reported Root Geotropic Activity*

COMPOUND NO	STRUCTURE	IAA TRANSMISSION AT 10μM		ROOT GEOTROPIC ACTIVITY (13
		cpm	%	(μM)
Ia		1792 ± 415	86	>100
Ib		1408 ± 358	68	100
Ic		438 ± 156	21	10
Id		355 ± 61	17	1
I		142 ± 121	7	0.01

to have auxin activity, as is shown by a consideration of the IAA molecule itself. The structural features necessary for auxin activity are therefore quite different from those required for auxin transport activity. There seems also to have been no previous attempt to describe the general requirements for auxin transport activity. With respect to the geotropic response, however, Jones *et al.* (12) had proposed earlier that an *o*-carboxyphenyl residue attached to a weighty cyclic nucleus was required. To the extent that the requirements for root geotropic activity are similar to auxin transport activity, our suggested rules may be regarded as a refinement of this proposition.

Comparison with TIBA and the Morphactins. It is to be noted that the auxin transport inhibitors TIBA IX[1] and the morphactins, *e.g.* chlorflurenol X, cannot be included in the correlation. TIBA appears to act at a different receptor site from the class described here because it acts directly on the auxin receptor site in corn coleoptiles while NPA III has a different receptor site (24).

The morphactins are also distinguishable because they have a variety of characteristic morphological effects (22) including structural changes to the root cap (21). These changes are not shown by either the propanedione IV or the pyrenoylbenzoic acid I (Geissler and Katekar, unpublished results), nor have they been reported for NPA III and DPX1840 V, R=OCH_3, which have been shown to inhibit cell elongation in the roots of *Pisum sativum* (9). It is known, however, that chlorflurenol X and NPA III compete for a common binding site in homogenates from corn coleoptiles (25), which would indicate a common mode of action. Since morphactins can be chemically degraded to fluorenones (23) and then to 2-phenylbenzoic acids (11) (which fulfil the chemical requirements of auxin transport inhibitors), and it is known that morphactins are rapidly degraded within the plant (22, 23), we speculate that the auxin transport-inhibiting properties of the morphactins may be due to their breakdown to 2-phenylbenzoic acids within the plant. This aspect is currently being investigated.

A common set of chemical properties for compounds which all affect a physiological process would indicate that they may act at a common binding site. Apart from the carboxylic acid function, the compounds have only a relatively inert and bulky residue, which would be consistent with the noncovalent binding to a lipid membrane fraction suggested for NPA III (24). The requirements for conjugation and aromaticity, together with the observed high activity for fused ring compounds (10, 15) would point to the possibility that the binding to active sites is in the nature of a noncovalent π-bonded interaction or charge-transfer complex. It may be that the fluoresceins II do not operate by a photodynamic mechanism (4), but rather that their biological activity is due to their other properties which fit them within the class.

Katekar (13) has pointed out that the suggested requirements for geotropic activity have predictive value because it is possible to design other molecules which would also conform to the requirements. This is equally possible for auxin transport inhibitors. For example, oxazoles and oxadiazoles of the types represented by XI and XII, together with the thiazole analogues, which have recently been claimed to be useful as herbicides and plant growth regulators (5), would also be expected to be auxin transport inhibitors, and their useful activities may well be due to this property. The extremely high activity of the more effective compounds which can show effects at concentrations as low as 10^{-3} μM (10, 13) prompts the speculation that they may mimic a naturally occurring plant growth substance. However, their structure-activity requirements do not appear to be related to any known hormone.

[1] Abbreviations: NPA: N-1-naphthylphthalamic acid III; TIBA: 2,3,5-triiodobenzoic acid IX; chlorflurenol: methyl-2-chloro-9-hydroxyfluorene-9-carboxylate X; DPX1840: 3,3a-dihydro-2-(*p*-methoxyphenyl)-8H-pyrazolo[5,-1a]isoindol-8-one V; R=OCH_3.

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