A New Class of Synthetic Auxin Transport Inhibitors

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ABSTRACT
Auxin transport inhibition by a new class of synthetic plant growth regulators, the 2-(3-aryl-5-pyrazolyl)benzoic acids, was examined in bean (Phaseolus vulgaris L.) using the donor-receiver agar cylinder technique. These compounds can be prepared by the dehydrogenation and ring cleavage of compounds like DPX-1840 (2-(4-methoxyphenyl)-3,3a-dihydro-8H-pyrazolo[5,1-a]isooindol-8-one) which was previously reported (Plant Physiol. 1972. 50: 322-327). These new growth regulators inhibit auxin transport more than DPX-1840 does as evidenced by their consistently greater reduction of basipetal auxin transport capacity in bean when incorporated into the receiver agar cylinder or applied foliarily to intact plants. Direct comparisons of the effect of DPX-1840, its dehydrogenation product (2-(4-methoxyphenyl)-8H-pyrazolo[5,1-a]isooindol-8-one), and its open-ring form (2-(3-(4-methoxyphenyl)-5-pyrazolyl)benzoic acid) on auxin transport indicated the following order of activity: ring-open > dehydrogenated form > DPX-1840. DPX-1840,14C, applied at 0.5 mg/l to etiolated bean hypocotyl hooks followed by extraction and thin layer chromatography, indicated the biological conversion of DPX-1840 to its open-ring form. Collectively, these results suggest that the biologically active forms of DPX-1840-type compounds are the open-ring (2-(3-aryl-5-pyrazolyl)benzoic acids.

A new class of synthetic plant growth regulators, the 2-(3-aryl-5-pyrazolyl)benzoic acids (III, IV) have been found. They can be derived chemically (4, 7) and biologically by the dehydrogenation and ring cleavage of compounds like DPX-1840 (2-(4-methoxyphenyl)3,3a-dihydro-8H-pyrazolo[5,1-a]isooindol-8-one) already shown to be a potent inhibitor of auxin transport (I).

Like DPX-1840 (I), these compounds also inhibit auxin transport and they modify plant growth in a manner which is consistent with this type of physiological action. Their morphological effects mimic those previously described for DPX-1840 including growth retardation, epinasty, breaking of apical dominance, loss of geo- and phototropic responsiveness (1), synergism with ethylene in abscission (1, 9), and induction of parthenocarpy (3, 10). These compounds are also readily taken up by leaves and roots and autoradiographs of plants treated with 14C-labeled DPX-1840 or IV clearly indicate symplastic movement within the plant.

In this paper we present data indicating that the primary physiological effect of these growth regulators is the disruption of auxin transport. We propose that the open-ring forms (e.g. III and IV) represent the biologically active metabolites of DPX-1840-type compounds.

MATERIALS AND METHODS
Plant Culture. Bean (Phaseolus vulgaris L. cv. Resistant Black Valentine and Pinto) plants were grown in a controlled environmental growth room (3200 ft-c; 18-hr photoperiod; temperature 24 C day, 19 C night; relative humidity 75 ± 3% day and night) in 15.2-cm plastic pots containing a peat moss-vermiculite mixture (Jiffy-Mix) mixed with sand (1:1, v/v) and were watered daily with Hoagland's nutrient solution.

Auxin Transport. The donor-receiver agar cylinder technique (2) was used to measure auxin transport capacity in 5-mm sections cut from the distal portion of the petiole of the primary leaf of bean 5 mm below the leaf blade. Two types of experiments were conducted. In the first type, the petiole sections were cut from Black Valentine beans and were placed with the distal end up on receiver agar cylinders (43.2 μl) containing 1, 10, or 100 μM of compounds I, II, or IV. Donor agar cylinders (43.2 μl) containing 2 μM mephathaleneacetic acid-1-14C (54.5 μCi/mmoll with a radiochemical purity of 97.8% as determined by paper chromatography (6) and liquid scintillation counting (5) were applied to the distal end of each petiole section for 4 hr in the dark at 30 C. Following transport the sections were cut in half, placed directly into a dioane scintillator fluid (8), extracted overnight on a shaker at 4 C, and counted. Donor and receiver agar cylinders were assayed similarly.

In the second type of experiment, the sections were cut in an identical fashion from Pinto beans 2 hr after foliar treatment with 1, 10, and 100 mg/l of compounds I, II, and III (3 ml/plant in acetone). Sections were placed on plain receiver agar cylinders, and auxin transport was measured as described above.

DPX-1840-14C Metabolism. Black Valentine beans were planted in vermiculite, watered with distilled H2O, and grown for 5 days in the dark at 28 C and 85% relative humidity. U-shaped hypocotyl hooks were cut into sections across the hook region directly above the attachment of the cotyledons and 15 g of heated (80 C, 2 min) and unheated hook tissue were incubated for 6 hr in the dark in 100 ml of 10 mm phosphate buffer (pH 5.5) containing 0.5 mg/l of DPX-1840-14C (14C in methoxy group, 4 μCi/mmoll). Following incubation, the tissue
was removed, rinsed, and immediately homogenized in cold 80% methanol for 4 min. The homogenate was centrifuged at 12,000g for 10 min, the methanol was removed under vacuum from the supernatant, and the aqueous phase was partitioned against diethyl ether at pH 8 to remove the DPX-1840-14C. The pH of the aqueous phase was then adjusted to 3 and re-extracted with ether to remove any ring-opened metabolite III derived from DPX-1840-14C. The ether extracts were reduced to dryness, and the residue was taken up in 0.2 ml of tetrahydrofuran and chromatographed on MN SIL G-25 u.v.-act TLC plates (Macherey-Nagel Co., Germany) in 1-butanol-ethanol-NH$_4$OH (2:1:1). The radioactivity profile of the chromatograms was determined by liquid scintillation counting of 0.5-cm sections from the TLC plates. The presence of metabolite III, in the pH 3 ether extract, was further confirmed by microesterification of the extract using BF$_3$-methanol reagent followed by TLC and high performance liquid chromatography.

**RESULTS**

DPX-1840 as reported (1) severely reduced the basipetal auxin transport capacity in bean petiole sections when incorporated into the receiver agar cylinder at 1, 10, and 100 μM (Fig. 1). However, the ring-open structures III and IV reduced auxin transport even more under identical conditions (Fig. 1). DPX-1840 reduced auxin transport 33, 62, and 79% at 1, 10, and 100 μM, respectively, while the corresponding values for III were 46, 67, and 90% and those for IV were 36, 69, and 92%. The ring-open structures III and IV appear to be more active auxin transport inhibitors since under our experimental conditions any differences in the activity of these compounds from variations in uptake, movement, and metabolism were minimized by directly treating the basal cut surface of the sections.

Similar results were obtained when intact plants were treated with 1, 10, and 100 mg/l of DPX-1840 (I), II, and III and auxin transport capacity was measured in petiole sections cut 2 hr later (Fig. 2). No significant differences were observed between DPX-1840 (I), II, or III at 1 mg/l but at 10 and 100 mg/l the order of activity was III > II > I. At 10 mg/l DPX-1840 (I) reduced the basipetal auxin transport capacity 20% while II and III reduced it by 50 and 60%, respectively. Similar but smaller differences were observed at 100 mg/l.

The differences in auxin transport inhibition 2 hr after foliar treatment (Fig. 2) correlated well with the growth retardation in similarly treated plants after 1 week (Fig. 3). At 1 ppm, where auxin transport was not significantly affected by I or III (Fig. 2), little growth retardation was observed, whereas, at the higher levels, where both significantly inhibited auxin transport, the plants were severely stunted. At 10 ppm, but not at 100 ppm, some regrowth occurred after 1 week with the weaker transport inhibitor DPX-1840 showing the greatest regrowth. Both I and III caused severe epinasty after 1 week at 10 and 100 ppm, but terminal leaf abscission occurred only at 100 ppm.

The chemical conversion of DPX-1840 (I) to the ring-open structure III has been described (4, 7). This conversion can also occur biologically (Fig. 4). Etiolated bean hypocotyl tissue converted DPX-1840-14C to the ring-open III at an average rate of 1 ng/g fresh weight·hr. This represents a minimum rate of ring opening of DPX-1840 within the tissue since there was considerable loss of 14C activity due to metabolism of the para-O14CH$_3$ group to 14CO$_2$ (12 ng/g fresh weight·hr) thus precluding the...
detection of ring-open structure IV. Very polar, aqueous-soluble $^{14}$C-labeled metabolites were also recovered from the DPX-1840-$^{14}$C treated tissue. Recent studies with $^{14}$C-labeled IV indicate the formation of two polar metabolites, which appear to be sugar and amino acid conjugates of the para-OH derivative of structure IV.

**DISCUSSION**

The 2-(3-aryl-5-pyrazolyl)benzoic acids are clearly more potent inhibitors of basipetal auxin transport than DPX-1840 and other data not reported suggest they are also more potent inhibitors of lateral auxin transport. The ability of bean hooks (Fig. 4) and other tissues (data not shown) to convert DPX-1840 to its ring-open form III coupled with the greater inherent auxin transport inhibition activity (Fig. 1) of III and IV suggests that the ring-open structures may be the active forms of their closed-ring counterparts. Several types of data support this view. First, the ring-open forms will qualitatively duplicate a wide range of different morphological effects caused by the closed-ring DPX-1840-type compounds. Second, these ring-open structures are generally more active on intact plants. For example, III and IV are several times more active in dwarfing soybeans than are the closed-ring compounds. However, since on intact plants uptake, movement and metabolism all affect the total plant response, the ring-open forms may not always be more active on intact plants even though they are inherently more potent inhibitors of auxin transport. For example, on cotton plants grown in a controlled environmental growth room ring-open IV applied foliarly was considerably less active than its dehydrogenated closed-ring counterpart. However, when the two were compared directly in receiver agar cylinder experiments, the reverse was true indicating that the ring-open form IV was the more potent auxin transport inhibitor. The activity in excised and whole plants was found to differ because the dehydrogenated closed-ring form was taken up by the cotton plant more readily than IV.

Another reason for believing the 2-(3-aryl-5-pyrazolyl)benzoic acids to be the biologically active forms is that heretofore DPX-1840 was unique in that it represented the only potent auxin transport inhibitor without a free carboxyl group. The potent auxin transport inhibitors TIBA (2,3,5-triodobenzoic acid), naptalam (N-1-naphthylphthalamic acid), and mephactin (methyl-2-chloro-9-hydroxyfluorene-9-carboxylate) all possess a free carboxyl group or an ester linkage which can easily yield a free carboxyl group. The ring-open form of DPX-1840 contains a free carboxyl group making the structural requirements for auxin transport inhibition uniformly similar in this respect. Perhaps this is not too surprising in view of the general structural requirement of a carboxyl group for auxin activity. Presumably, this structural similarity between auxin transport inhibitors and auxins reflects in some way the mechanism of action of these inhibitors.

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**LITERATURE CITED**