Auxin Activity of Substituted Benzoic Acids and Their Effect on Polar Auxin Transport

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Summary. Six dichloro-, 3 trichloro-, 2 triodo-, and 3 heterosubstituted benzoic acids (amiben, dinoben, dicamba), and N-1-naphthylphthalamic acid have been tested for effects on growth and on polar auxin transport. Growth activity with and without kinetin was measured by effects on fresh and dry weights of 30-day cultures of fresh tobacco pith. Transport inhibition was measured by following uptake and output of IAA-2-14C through 10 mm bean epicotyl sections. The distribution of callus growth on vascularized tobacco stem segments was also observed. *Avena* first internode extension assays established the relative activities: dicamba > amiben > dinoben suggested by pith growth results. Growth effects of active compounds were similar with and without kinetin, except that amiben was less active with kinetin, while 2,3,6-trichlorobenzoic acid was more active with kinetin than alone. The weak auxin activity of NPA was confirmed. Transport experiments showed that NPA was the most inhibitory compound tested, followed by TIBA. Other compounds tested were at least 300 times less inhibitory to IAA transport. The best growth promoters were the least inhibitory to transport, and the most effective transport inhibitors were at best poor auxins. It is suggested that the weak auxin and auxin synergistic activity of TIBA (and perhaps 2,3-dichlorobenzoic acid) in extension growth tests arises from its inhibition of transport of endogenous or added auxin out of the sections, rather than from its intrinsic auxin activity. Chemically induced apolar callus growth on vascularized tobacco stem explants can arise from inhibition of native auxin transport, apolar growth stimulation by auxinic action of the test compound, or both.

Prior studies (10, 11, 20, 22, 23, 24, 25, 32) of the effect of chemicals on polar auxin transport have shown that those which A) interfere with ATP formation (*DNP*), B) are sulfhydryl binders (*TIBA*, iodoacetate, PCMB) or C) are weak (NPA, 2,5-dichlorobenzoic acid) or strong (2,4-D) auxins can all decrease the amount of IAA reaching a receptor block from the basal end of a section of stem tissue to whose apical end it has been applied. A limited survey of 10 phenoxyacetic acids (24) showed that transport inhibition increased with the number of substituted chlorine atoms up to 3, and that the active molecules tended to have higher absorbability (to charcoal). No direct correlation with growth promotion was sought. In an earlier study (16) it was established that the polar growth of callus on tobacco stem explants containing vascular tissue arises from the accumulation at the basal end of continuously basipetally transported growth factors, and a variety of substituted benzoic acids and related compounds were tested for their effects on callus distribution. Although several compounds showed activity in causing apolar callus growth, the question remained open whether the main effect was on the transport of endogenous auxin or was a non-polar stimulation of callus growth by the test compound itself.

Determination of the relative growth promoting and transport inhibiting capacities of the benzoic acid series seemed warranted, to distinguish between effects on growth and on auxin transport as explanations for the apolar callus growth on tobacco explants observed in previous experiments (16), and to broaden the base for correlations between growth activity and effect on auxin transport generally. NPA was included in the survey to check its reported weak auxin activity (19, 20, 30) by an independent test system, and to provide quantitative

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1. This study was aided by funds from the National Science Foundation, Grants C 14545 and GB 153.
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3. The following abbreviations and trivial names are used throughout: *DNP* = 2,4-dinitrophenol, NPA = N-1-naphthylphthalamic acid, PCMB = p-chlorophenoxymercuribenzoate, *TIBA* = 2,3,5-triiodobenzoic acid, amiben = 3-amino-2,4-dichlorobenzoic acid, dicamba = 2,5-dichloro-6-methoxybenzoic acid, dinoben = 2,5-dichloro-3-nitrobenzoic acid.
data on its ability to inhibit IAA transport. As neither the mode of action of auxins on growth nor their method of transport are clear, evidence bearing on a possible relation between the 2 is useful. We report here the effects of 14 substituted benzoic acids and NPA on 1) growth of tobacco pith cultures with and without kinetin, and 2) the transport of radioactive IAA through bean epicotyl sections.

The pith tissue was selected in order to relate the growth activity to earlier experiments on tobacco, and to test whether the promotion of cell enlargement by the test compounds alone was constantly correlated with callus growth with cell division when kinetin was present. Weakly auxinic compounds might be expected to have a greater activity with kinetin than without. Bean epicotyl tissue was selected for the transport studies because it is easy to obtain from uniform seedlings, is not hollow (as are hypocotyls), and thus does not present an inner surface along which auxin might move by diffusion.

**Materials and Methods**

**Growth Experiments. Plant Material.** Pith sections were taken from the stems of greenhouse-grown tobacco (*Nicotiana tabacum* L. var. Wis. Havana 38) plants about 3 feet tall. A No. 2 cork borer fitted with a plunger was used to remove cores of pith from the center of the stem, and a double-bladed cutter was used to obtain sections of equal length. Aseptic stem explants were obtained by cutting slabs tangentially from cylinders of stem from which the outer tissues down to the cambium had been peeled away (18). These were tested to gauge the effect of chemicals on callus redistribution.

**Sources of Compounds.** Bought from Eastman: 2, 4- and 3, 4-dichlorobenzoic, TIBA; from K & K Laboratories, 3, 4, 5-triodobenzoic; from Aldrich Chemical Company, Incorporated, 2, 6-dichlorobenzoic. Gifts of all but 2 of the remaining compounds are acknowledged later. The methods of synthesis of the remaining 2 are summarized below.

2, 3-Dichlorobenzoic: The method of Hope and Riley (14) was modified. Diazotization of 2-amino-3-nitrotoluene (Aldrich) followed by chlorination with Cl₂ yielded 2-chloro-3-nitrotoluene. This was reduced with SnCl₂ and the diazotization and chlorination steps repeated. The resulting 2, 3-dichlorotoluene was oxidized with K₂Cr₂O₇ to yield 2, 3-dichlorobenzoic acid (m.p. reported: 164-168.3°; found: 164-167° uncorr.).

2, 5-Dichlorobenzoic Acid: The method of Bornwater and Holleman (6) was followed. Diazotization and chlorination of 3, 5-diaminobenzoic acid (Eastman) yielded 3, 5-dichlorobenzoic. The crystals obtained from an alcohol-water mixture melted at 182 to 184° uncorr. (literature value 188°).

The other compounds were used as received except that TIBA was recrystallized from an alcohol-water mixture and the NPA sodium salt was converted to the free acid by the addition of HCl and recrystallized from an acetone-water mixture.

**Media.** A modified White's medium (18) served as the basal medium. A set of 4 treatments to check the tissue response was run in every experiment: A) basal control, B) IAA at 11 μM (2 mg/liter), C) kinetin at 0.9 μM, (0.2 mg/liter) and D) these respective levels of IAA and kinetin in combination. The test compound was added to the basal medium at 1000, 100, 10, and 1 μM, either alone or with 0.9 μM kinetin. Pith sections were grown on each of the above media. In addition, stem explants were grown on basal medium and on the 3 highest levels of test compound.

At first, each compound was titrated to pH 6.0 and added to the media prior to autoclaving for 16 minutes at 120°, except NPA, which was filter sterilized and added aseptically to the autoclaved basal medium. Later it became necessary to check the assumption that the substituted benzoic acids would be stable to autoclaving and each compound was tested at least once by the filter sterilization method. The results were essentially the same as those from the earlier experiments, except for dicamba, amiben and dinoben. Dicamba was seriously inactivated by autoclaving, amiben moderately, and dinoben slightly, as will be seen below.

Standard 4 ounce square infant nursing bottles were used as culture vessels. It was found that the 2-piece plastic nipple tops can be sealed into 1 unit with a soldering gun to provide a reusable cap which has no removable inner liner. Each bottle contained 50 ml of medium. Five bottles of each treatment were run. Three pith sections or 3 stem explants were placed in each bottle.

Fresh weights and dry weights of pith sections were measured after 30 days in culture. Representative stem explants were photographed after fresh weights had been recorded, to allow evaluation of the polarity of callus distribution. The contents of each bottle were weighed as one and statistics were based on the number of bottles. Each compound was tested in at least 2 experiments unless otherwise noted.

**Extension Growth Tests.** Avena first internode extension assays of 3 benzoic acid derivatives were carried out essentially according to the procedure of Crosby et al. (8). Forked deer oats were used, and a plexiglas cutting guide and double razor blade cutter enabled the removal of uniform 4 mm sections 3 mm below the node.

**Transport Experiments. Plant Material.** Seeds of *Phaseolus vulgaris* L. var. Kentucky Wonder were allowed to imbibe water overnight, planted in vermiculite, and grown in the greenhouse for 7 to 9 days, at which time the primary leaves had expanded and a few cm of shoot had grown above the primary node. Sections 9.3 mm long were taken beginning 3 mm below the primary node by means of a double-bladed cutter and cutting guide. An
India ink mark was placed near the apical end before cutting.

**Section Holders.** Plastic jigs were constructed to hold a 20 mm diameter, 0.5 ml receiver disc of 1.5% agar, and 20 bean epicotyl sections. An 0.5 ml donor disc containing 4 μg/ml of IAA-2-14C (sp. act. 2 c/mole, New England Nuclear) in 1.5% agar was placed on top of each set of 20 sections, and the assembly placed in a moist chamber to allow transport. A drop of water was spread on the receiver block before the sections were placed in the holder, and a small drop of water was put on the apical end of each section before the donor was applied, to assure good contact.

**Counting Procedure.** After the transport interval the agar discs were separated from the tissues and prepared for counting in either of 2 ways. In the initial experiments a thin-window proportional gas flow counter (Baird-Atomic Model 750) was used; the agar discs were dried on glass cover slips 22 mm in diameter at 90° for 20 to 30 minutes and the cover slips placed on standard counting planchets. Subsequently, a Packard Tri-Carb Model 4300 scintillation counter became available; the samples were prepared by melting the agar disc at 100° in an empty counting vial, then adding warm scintillation fluid (ca 60°), capping, and agitating the vial for a few seconds on a Vortex mixer to assure homogeneous distribution. The scintillation mixture contained 30 g/l Cab-O-Sil (to disperse the aqueous phase), 4 g 2,5-diphenyloxazole (PPO), and 50 mg 1,4-bis-2-(4-methyl-5-phenyloxazolyl)-benzene (dimethyl POPCP), in 1 liter of toluene. The counting efficiency of the scintillation system was 10 times greater than that of the gas flow system.

**Experimental Plan.** The compounds to be tested were incorporated in the receiver blocks in order to make it more likely that their effect would be upon transport, not uptake. The compounds would have had to diffuse through 10 mm of tissue, unlikely in 4 hours. Both donors and receivers were counted after transport had occurred, and in no case could the decreased output be related to a proportional decrease in uptake. Hence inhibition of uptake was ruled out as the mode of action of these compounds. Prior to using the jigs, we attempted to apply the compounds in a ring of lanolin around each section. This held the sections together, and prevented any film of surface moisture from transferring radioactivity by diffusion. However, the compounds had to penetrate the epidermis rather than severed parenchyma cells, and hence entry of the inhibitor could have been variable. Moreover, the method was unduly tedious. Even so, several useful experiments were done in which the optimum concentrations of agar and of IAA were determined. Several of the compounds were tested in this way, also; the results agreed with those obtained with the improved technique and are not further reported here. That the sections do not transport auxin by external moisture films is shown by the irreversibility of transport upon inversion of the tissue (fig 1B). There is a modest error introduced by the fact that the sections do elongate during the transport interval, and if they vary greatly in their rate of elongation, the donor may lose contact with the slower growing ones. The general reproducibility of the results argues that this error is not a serious one under our conditions.

**Results**

**Growth Experiments.** The pith growth data are summarized in table I, which gives the concentrations which stimulated growth beyond that occurring on basal (without IAA or kinetin) medium (significant at 95% confidence limits). Zeros indicate inactive or merely inhibitory compounds.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Fr wt</th>
<th>Alone</th>
<th>Dry wt</th>
<th>Fr wt</th>
<th>With kinetin</th>
<th>Dry wt</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,3-dichlorobenzoic</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2,4-&quot;</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2,5-&quot;</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2,6-&quot;</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3,4-&quot;</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3,5-&quot;</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>0</td>
</tr>
<tr>
<td>2,3,5-trichlorobenzoic</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2,3-5-&quot;</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>0</td>
<td>100:10,1</td>
</tr>
<tr>
<td>2,4,6-&quot;</td>
<td>0</td>
<td>0</td>
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<td>0</td>
</tr>
<tr>
<td>2,3,5-trioleobenzoic</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3,4,5-&quot;</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3-amino-2,5-dichlorobenzoic</td>
<td>1000-1</td>
<td>1000-1</td>
<td>1000-1</td>
<td>1000-1</td>
<td>1000-1</td>
<td>1000-1</td>
</tr>
<tr>
<td>2,5-dichloro-3-nitrobenzoic</td>
<td>1000-1</td>
<td>1000-1</td>
<td>1000-1</td>
<td>1000-1</td>
<td>1000-1</td>
<td>1000-1</td>
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<tr>
<td>2,5-dichloro-6-methoxybenzoic</td>
<td>100-1</td>
<td>1000-1</td>
<td>1000-1</td>
<td>1000-1</td>
<td>1000-1</td>
<td>1000-1</td>
</tr>
<tr>
<td>NPA</td>
<td>100</td>
<td>0</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Some of the compounds active at low levels were inhibitory at the highest level tested, 1000 μM.

Of the 6 dichlorobenzoic acid isomers only 2,6-dichlorobenzoic stimulated fresh weight when supplied alone (100 μM); no increases were found in the presence of kinetin. Other workers (21,27) have found weak auxin activity for this compound.

Among the 3 trichlorobenzoic acids tested, 2,3,6-trichlorobenzoic caused a fresh weight increase alone at 1000 μM, and both fresh and dry weight increases at 100 μM and 100-1 μM respectively in the presence of kinetin. A dry weight increase was caused by 10 μM 2,4,5-trichlorobenzoic in the presence of kinetin. These results are consistent with the known strong auxin activity of 2,3,6-trichlorobenzoic acid (5,27); however, the weak activity of 2,4,5-trichlorobenzoic is harder to account for, and may be fortuitous. Substitution of chlorine in the 4-position of benzoic acids usually renders them inactive or toxic.

Neither 2,3,5- nor 3,4,5- triiodobenzoic acid stimulated growth alone or with kinetin. This result is felt significant and is discussed further in the next section.

The heterosubstituted herbicides, amiben, dinoben, and dicamba, all showed auxin activity by themselves and with kinetin. Dicamba appeared to be the most active, stimulating with kinetin only at the lowest levels tested (10 and 1 μM). On the basis of the magnitudes of the responses, amiben was more active than dinoben.

NPA showed very weak auxin activity at 100 μM alone, and at 10 μM in the presence of kinetin. The effects were on fresh weight only. These results agree with the reported weak auxin activity previously reported for NPA in Avena curvature (20) and straight growth (30) tests.

Avena first internode assays of amiben, dinoben and dicamba were carried out to confirm the relative activities of these compounds as suggested by the pith data. Table II compares their activities with that of IAA. The maximum response to each herbicide is about the same, and amounts to 80% of the IAA value. But the concentrations required to achieve maximum elongation are 1 μM for dicamba (the same as for IAA), 10 μM for amiben, and 100 μM for dinoben. Hence they may be ranked dicamba > amiben > dinoben, in agreement with the pith growth data.

The polarity of callus growth on tobacco stem explants in response to some of the compounds is shown in Table III. Among the dichlorinated benzoic acids, only the 2,4- and 3,4- isomers were without effect. The 2,3- isomer was weakly active, whereas the 2,5-, 2,6- and 3,5-derivatives had a greater effect. There is thus some callus redistribution caused by both active and inactive compounds with respect to growth promotion, in this series. The activity of 2,6-dichlorobenzoic in causing apolar callus growth has been reported previously (16).

The active auxin 2,3,6-trichlorobenzoic acid caused highly apolar callus growth in these experiments, in agreement with prior studies (16), whereas the 2,3,5-derivative did not affect either growth or callus distribution.

In contrast, the 2,3,5-triiodo- derivative, TIBA, was as in previous work (16,25), highly effective in causing apolar callus growth.

Of the 3 heterosubstituted herbicides, dinoben caused the least callus redistribution, followed by amiben and dicamba. The relative strengths are rated on the basis of the degree of apolarity and the concentration needed to bring it about: the

Table II. Elongation of Avena First Internode Sections Versus Concentrations of Three Benzoic Acid Derivatives and IAA

Maxima are italicized.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>IAA</th>
<th>Log concentration of</th>
<th>Amiben</th>
<th>Dinoben</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>-7</td>
<td>-6</td>
<td>-5</td>
<td>-6</td>
</tr>
<tr>
<td>ΔL (mm)</td>
<td>0.73</td>
<td>2.15</td>
<td>2.69</td>
<td>1.50</td>
<td>1.48</td>
</tr>
<tr>
<td>ΔL as %</td>
<td>100</td>
<td>294</td>
<td>360</td>
<td>237</td>
<td>204</td>
</tr>
<tr>
<td>control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
effects on callus distribution are correlated with the auxinic strength of these compounds.

Transport Experiments. The Transport System.
Prior to testing the compounds, several aspects of the transport system were studied. The rate of both acropetal and basipetal IAA uptake from the donors is shown in figure 1A, and output into receivers in figure 1B, over a 24-hour interval. On the basis of these data, 4 hours was chosen as the standard interval for tests of compounds for transport inhibition. It allowed enough radioactivity to come through to count conveniently, but did not yet represent a maximum value and should therefore be sensitive to small effects.

Figure 1B shows the degree of polarity of transport in this tissue. Radioactive IAA applied to the apical ends moved through rapidly, appearing in the receiver after 40 to 80 minutes. When similar donor blocks were applied to the basal ends of inverted sections, no counts above background were measured until 24 hours, by which time diffusion could have accounted for the observed amount.

Table IV illustrates that the transport system can be saturated in this tissue. If the donor concentration is increased 5-fold, the receiver concentration at best is only about doubled after 5 hours. Yet the rate of uptake from the donor to the tissue does increase as the donor concentration goes up since the percent entering the tissue per unit time is nearly the same in each case. It would appear that at 4 µg/ml, the transport system is not fully loaded, and thus this was taken as a suitable concentration for testing inhibitors.

Short-term measurements of transport (fig 2) indicated that the amount of IAA appearing in the receiver increases approximately exponentially with time during the first 2 hours; it is difficult to extrapolate back to a clear-cut time at which the auxin front appears.

Effects of Compounds. All 6 dichlorinated benzoic acids were tested, and their effects are shown in figure 3. These compounds were tested in pairs with 1 set of untreated controls for each pair. The

Table IV. Relation of Uptake and Output of Radioactive IAA to Donor Concentration
Bean epicotyl sections were 10 mm long; transport interval was 5 hours.

<table>
<thead>
<tr>
<th>Expt</th>
<th>Donor conc µg/ml</th>
<th>c/m in donor</th>
<th>Uptake</th>
<th>Output</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
<td>c/m</td>
<td>% of donor</td>
</tr>
<tr>
<td>T-52</td>
<td>20</td>
<td>15,800</td>
<td>9500</td>
<td>6300</td>
</tr>
<tr>
<td>T-56</td>
<td>4</td>
<td>2725</td>
<td>1740</td>
<td>985</td>
</tr>
</tbody>
</table>
results are expressed as percent inhibition of the control transport rate to enable comparisons, since the control values did not vary greatly. Although a perfect progression in activity was not found, certain clear differences emerged. The 2,3- isomer was the most inhibitory, followed by the 3,5-. Least inhibitory to transport was 2,6-dichlorobenzoic. The 2,4-, 3,4-, and 2,5- isomers formed an intermediate group, some inhibiting more at low concentrations and less at the higher ones, thus making it hard to consider the concentration giving 50% inhibition to be a simple and adequate criterion of activity. Considering relative inhibition at 100 μM concentration, we can rank these compounds in approximate order of decreasing inhibition as follows: 2,5- > 3,5- > 2,4- > 3,4- > 2,6-. The relation of these data to growth data is considered in the Discussion section below.

The effects of various trisubstituted benzoic acids, 2,4-D, and NPA are compared in figure 4. Whereas concentrations generally toxic to the tissue were required before severe transport inhibition was caused by most of the benzoic acids and 2,4-D, very low concentrations of TIBA and NPA were highly effective in blocking transport. Again it was not possible to rank the less active compounds in strict order of decreasing transport inhibition by any such simple criterion as the concentration giving 50% inhibition, for the reason given above. For example, dinoben inhibited transport more than amiben at 0.1 to 10 μM, whereas above 10 μM they had very similar activity. Of the 2 trichlorinated benzoic acids tested, 2,3,5- inhibited transport more than did 2,3,6-. The response to dicamba resembled that to 2,3,6-trichlorobenzoic acid, and amiben and was slightly more inhibitory.

The dosage-response curve for 2,4-D was essentially the same as that reported by Niedergang-Kamien and Leopold (24) for this compound in a sunflower test system, assayed by the *Avena* curvature test. The significance of this resemblance is questionable, however, because their dosage/response curve is based on an uncorrected *Avena* curvature test; the slight inhibitory effect of 2,4-D in the receiver blocks on the curvature test itself was not taken into account. When corrections were made, 2,4-D at 0.5 mM inhibited transport 13 to 20%. In our experiments, the inhibition at this concentration was about 70%. The difference may have been due as much to the tissues as to the methods of measurement.

For unknown reasons it was difficult to get reproducible results at the lowest level of NPA tested, 0.1 mM. The value shown on the curve is the mean of all values obtained, but is not statistically different from the controls. The inhibitions at higher levels are significant. Hence this compound has truly remarkable transport-inhibiting qualities.

On the basis of the curves in figure 4 one can thus rank these compounds in the following approximate order of decreasing transport inhibition: NPA > TIBA > > > > > 2,3,5-trichlorobenzoic acid > dinoben, 2,4-D > amiben, dicamba, 2,3,6-trichlorobenzoic acid.
Discussion

Growth Experiments. These data are of interest from 2 viewpoints, discussed in order below.

Differential Effects With and Without Kinetin. The most active auxins (2,5-dichloro-6-methoxy-; 3-amino-2, 5 dichloro-; 2, 5-dichloro-3-nitro-; and 2, 3, 5-trichlorobenzoic acids) promoted growth with cell division in the presence of kinetin as well as cell enlargement when given alone. Both 3-amino-2, 5-dichlorobenzoic and 2, 6-dichlorobenzoic acids appeared to be less effective relative to IAA when kinetin was present than when they were supplied alone. The reverse situation appeared to obtain for 2, 3, 6-trichlorobenzoic, which was active at lower concentrations with kinetin than when given alone. The other active compounds had about the same relative activity with kinetin as without.

Hicks et al. (13) have reported a survey of all the mono- and di-chlorinated benzoic acids, as well as 14 mono-, di-, and tri-substituted phenoxyacetic acid derivatives, and the respective parent compounds, for their effects, with and without kinetin, on both cell expansion and calcium-induced cell division in Jerusalem artichoke tuber tissue (2). Of the dichlorinated benzoic acids, they found that the 2, 3-, 2, 5- and 2, 6- isomers were the most active; of all the compounds tested, those which stimulated cell enlargement also promoted growth with cell division. Quantitative differences up to several fold were seen in the effectiveness of stimulation of the 2 processes, but the data were not felt sufficiently precise to warrant emphasis of this point (Setterfield, personal communication). Their assay consisted of short term (24-48 hour) experiments and determination of the mitotic index as well as rate of fresh weight increase. Our longer term experiments may have been more sensitive to cumulative small effects.

If these differences in the effectiveness of stimulation of enlargement versus division are not merely reflections of the variability of the test systems, they suggest that the role of auxins may be different in the 2 cases. Inasmuch as the biochemical roles of auxins and cytokinins remain to be clarified, only further experiments can decide the matter.

Agreement of Structure/Activity Data with Those of Other Workers. The only dichlorinated benzoic acids reported active in the slit pea test and other assays (13, 21, 27) are the 2,3-, 2,5-, and 2,6- isomers. The 2,5- derivative seemed to be the most active. In our hands the 2,6-isomer gave the greatest growth response, while 2,3- and 2, 5-failed to stimulate pith growth, though they did cause non-polar growth of callus on vascularized stem tissue.

The auxin activity of 2, 5-dichloro-6-methoxybenzoic acid found here agrees with its selective herbicidal effects on broadleaved plants (3) and with Thimann's observation (private communication) that it is a strong auxin, about as active as IAA in the *Avena* coleoptile extension test, and 3 to 4 times as active in the slit pea test. The auxinic action of amiben and dinoben found here is consistent with the data of Sutherland et al. (29), who found that both compounds caused formative effects in soybeans, and with the widely reported effectiveness of both compounds as selective herbicides against broadleaved plants. Baker (4) tested amiben in the *Avena* coleoptile extension test and found it had the same effect at 100 μM as did IAA or 2, 4-D at 10 μM. Our results agree closely.

The failure of TIBA to promote pith growth confirms the data of Niedergang-Kamien and Skoog (25) for stem segments containing vascular tissue, but is at variance with its reported synergistic effect on low levels of endogenous auxin (31) or added IAA (1, 31), and its reported stimulation of section growth (21). These differences can be accounted for on the basis of the strong inhibition by TIBA of polar auxin transport. In stem or coleoptile elongation tests, the tissues are seldom totally depleted of endogenous auxin during the entire growth period. This auxin is continually being polarly transported out the basal end of the section and diluted in the surrounding medium. If a strong transport inhibitor such as TIBA should prevent the exit of auxin, its concentration in the tissue would increase and greater extension would ensue.

Support for the concept that auxin continuously flows through stem sections floating in solutions of IAA, and thus by inference that endogenous auxin would be continuously excreted, is found in the results of Hertel and Leopold (12), who showed that short sections of *Zea* coleoptiles floated on solutions of radioactive IAA accumulated more 14C when TIBA was also present in the medium. They interpreted the data as showing TIBA inhibition of basal IAA secretion, but not apical uptake.

The failure of 2, 3-dichlorobenzoic acid, also active in extension assays, to stimulate growth of tobacco pith might be explained on the same basis, inasmuch as we have found it to be the most potent inhibitor of polar transport among the dichlorobenzoic acids. The failure of 2, 5- dichlorobenzoic to stimulate pith growth is less clear on this basis, because it was rather less active as a transport inhibitor than 2, 3-dichlorobenzoic. Moreover, both these compounds were active in the Jerusalem artichoke tuber tissue system (13), which presumably was not susceptible to influences on polar auxin transport.

Transport Experiments. There are 2 features of the transport system in bean epicotyl which warrant fuller consideration in another context, hence will be only mentioned here. The first is that there seemed to be no sharp front of auxin activity moving into the receptor blocks. Rather, output initially was approximately exponential, suggesting...
that the distribution of IAA-\(^{14}\)C in the tissues declines logarithmically from the source, as one might expect if complexing or destruction were to remove some of the auxin from the transport stream at each cell (15). The second feature is that after about 8 hours the concentration in the receiver again decreases (fig 1B). Each point on the time curve was determined by counting receptor blocks which had been in continuous contact since time zero, hence the decrease must represent either re-entry of the radioactivity into the basal ends of the sections or degradation of the auxin by plant enzymes or by bacteria, with release of the \(^{14}\)CO\(_2\) into the atmosphere. The later explanation is doubtful for 2 reasons; First, the IAA was labelled in the 2-carbon atom of the side chain, which is not readily lost upon oxidation, and second, the rate of decrease is greater between 8 and 12 hours than between 12 and 24 hours. This latter portion of the output curve roughly parallels the initial uptake curve from the donors (fig 1A), the rate depending on the donor concentration. If bacterial degradation were occurring, one would expect the rate of decline to increase with time as the organisms multiply.

Although the above observations suggest that some metabolic interconversion of IAA takes place within the stem sections, all of that fraction of the radioactivity which entered the receptor blocks appeared upon chromatography to be unaltered IAA (unpublished results from this laboratory). Pilet's demonstration (26) that radioactive IAA in Lens epicotyl sections is pushed out by unlabeled IAA applied subsequently to the apical ends, and conversely, unlabeled IAA in the tissues moves into the receptors ahead of a radioactive IAA chaser, and that TIBA inhibition of output results in decreased uptake, suggests the amount leaving the donor is apparently determined by the rate at which not only destruction or complexing occurs but also transport operates, the auxin concentration gradient between donor and tissue being influenced by both systems.

**Structure/Activity Relations.** Upon comparing growth promoting and transport inhibiting activity of the compounds tested, one finds that in general the most active auxins (2, 3, 6-dichloro-, 2, 3, 6-trichloro-, 2, 5-dichloro-6-methoxy-, and 3-amino-2, 5-dichlorobenzoic acids, 2, 4-D) are the least active transport inhibitors. Conversely, NPA, by far the most active transport inhibitor tested, is at best a very poor auxin, and TIBA, another potent transport inhibitor, is inactive in the pith growth assay. Both 2,3- and 3, 5-dichlorobenzoic acids, the most inhibitory to transport of the dichlorinated benzoic acid series, were also inactive in the pith growth test, although they both caused some redistribution of callus growth on vascularized tobacco stem explants.

These data support the contention of Niedergang-Kamien and Skoog (25) that the chief mode of action of TIBA and some other weak auxins at low levels is on auxin transport rather than on growth itself. Both TIBA (11, 12, 16, 17, 22, 23, 25, 26, 32) and NPA (19, 20) have previously been found to be strong inhibitors of transport; the present results show quantitatively the remarkable extent to which this is true.

It has frequently been assumed (20, 22, 24) that transport inhibitors function by competing with auxins for sites on membrane carriers (9). If they do, then the molecular specificity for attachment to the carriers would appear to differ from that needed for growth promotion, otherwise one would expect that the more auxin-like a molecule, the better it would compete for sites on the carrier system. The only kinetic data available are those of Christie and Leopold (7), who found that TIBA seemed to inhibit IAA uptake competitively but secretion non-competitively. Further work is needed to decide whether auxin transport inhibitors compete with IAA for carrier sites, alter the properties of the sites so as to inactivate them, or involve some other mechanism, such as altered IAA metabolism.

**Conclusion**

1. TIBA exerts its effects on growth through IAA transport inhibition. Its reported weak auxin and auxin synergistic actions can be accounted for in terms of effects on the transport of endogenous auxin. 2. The response of tobacco pith to a variety of auxins is essentially the same as that of Jerusalem artichoke tissue, in that compounds which stimulated cell enlargement also stimulated growth with cell division. Certain compounds showed minor differences in their effect on the 2 kinds of growth. 3. Apolar callus growth in vascularized tobacco stem segments can result from chemical inhibition of transport of endogenous auxin, a non-polar stimulation of growth by an auxinic compound, or both. 4. The most active transport inhibitors have little or no growth promoting action; the best auxins are least inhibitory to auxin transport.

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