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Inhibition of Auxin Transport by Isoquinolinedione Herbicides

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Abstract. 2-(*p*-carbethoxyphenyl)-1,3(2H,4H)-isoquinolinedione (CEPIQ), an experimental herbicide, caused effects on geotropism, which are often indicative of an effect on auxin transport, in a whole plant herbicidal screen. However, it showed little or no activity in an in vitro binding assay in corn coleoptiles for the auxin-transport inhibitor, *N*-1-naphthylphthalamic acid (NPA). Other active isoquinolinedione analogues of this compound did, however, exhibit significant in vitro activity. Direct measurements of auxin transport in corn coleoptiles were undertaken in an attempt to resolve the apparent discrepancy between herbicidal and binding activities. In all cases examined, compounds that were highly active on whole plants were good inhibitors of auxin transport, and compounds that were weak as herbicides showed little or no effect on auxin transport. Therefore, it is concluded that the mode of action of these isoquinolinedione herbicides is the inhibition of auxin transport. Ring-opened analogues of several isoquinolinediones were synthesized and assayed in both the transport and binding assays, in order to test whether compounds in this class express their herbicidal activity by undergoing ring-opening in vivo, yielding products that are more straightforward analogues of NPA with free carboxyl groups. The homophthalamic acids had little or no activity in both assays. On the other hand, the *p*-ethyl- and *p*-ethoxy-phenyl phthalamic acids showed auxin transport inhibition comparable to the parent isoquinolinediones, but with markedly increased binding activity. These results support the possible role of ring-opening in the generation of biological activity. However, the *p*-carbethoxyphenyl phthalamic acid, analogous to CEPIQ, was very weak in both assays. Thus, ring-opening in vivo cannot alone account for the biological activity of this class of compounds.

For over a decade, 2-substituted-isoquinolinedione plant growth regulators have been known to cause stature reduction, axillary bud development, and altered canopy in soybeans (D'Amico 1978). When an analogue of these compounds, 2-(*p*-carbethoxyphenyl)-1,3(2H,4H)isoquinolinedione (CEPIQ), was first tested in a whole plant phytotoxicity screen, striking among its effects was the ageotropic behavior of the roots of several test species. Since interference with root geotropism is characteristic of inhibitors of auxin transport, it was presumed that this compound, too, was acting via that mode of action. Most auxin-transport inhibitors bind to a specific site on the plant plasma membrane, which is defined by its affinity for the herbicide naptalam, *N*-1-naphthylphthalamic acid (NPA) (Thomson et al. 1973). However, when CEPIQ was tested in an in vitro NPA-binding assay, it showed little or no activity.

Subsequently, a synthesis program was carried out to prepare novel isoquinolinedione analogues of CEPIQ. Among the most active of these compounds in the whole plant screen were those with alkoxycarbonyl-, halo-, alkyl-, or alkoxy-substituents at the *para*- and/or *meta*-positions on the phenyl ring. Paradoxically, some of these active compounds showed quite good in vitro activity in the NPA-binding assay, whereas others, like CEPIQ, were poor competitors with NPA. In an attempt to resolve this paradox of a lack of correlation between in vitro and whole plant activity, direct measurements of auxin transport through corn coleoptiles were carried out. These results are reported here, along with possible explanations for

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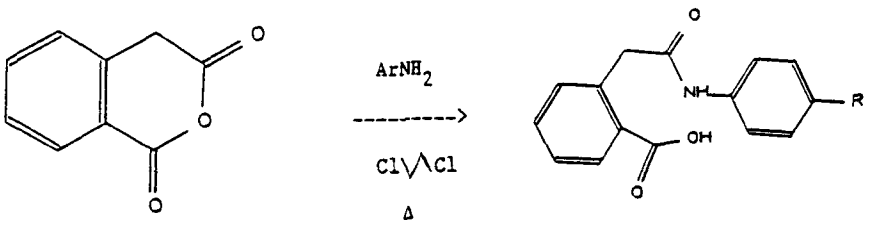


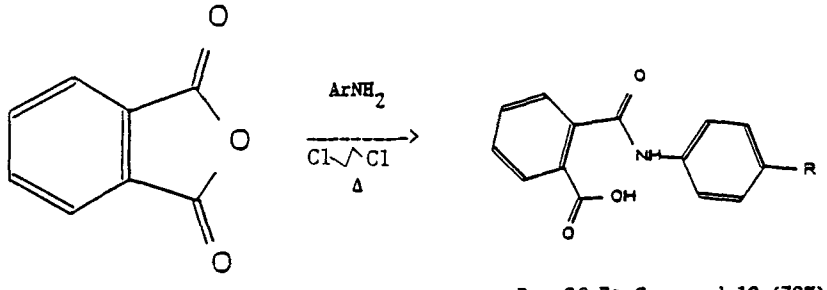
R = CO_2Et Compound 8 (41%)

R = Cl Compound 11 (51%)

R = OEt Compound 14 (76%)

R = Et Compound 17 (40%)

Scheme 1. Synthesis of homophthalamic acids.



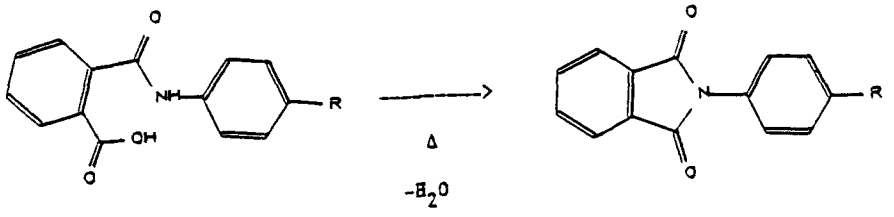
R = CO_2Et Compound 10 (70%)

R = Cl Compound 13 (32%)

R = OEt Compound 16

R = Et Compound 19 (50%)

Scheme 2. Synthesis of phthalamic acids.



R = CO_2Et Compound 10

R = Cl Compound 13

R = OEt Compound 16

R = Et Compound 19

R = CO_2Et Compound 9 (88%)

R = Cl Compound 12 (53%)

R = OEt Compound 15 (70%)

R = Et Compound 18 (80%)

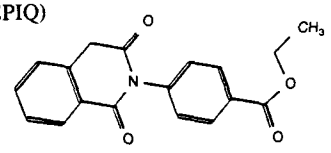
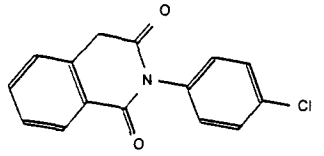
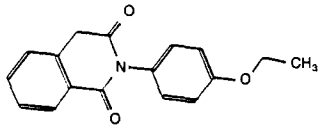
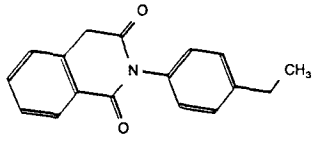
Scheme 3. Synthesis of N-(*p*-substituted)phenylphthalimides.

the apparent discrepancy between herbicidal and binding activities.

Materials and Methods

In vitro activities of isoquinolinedione analogues were compared by measuring their ability to displace [^{3}H]NPA from its binding site on membranes from etiolated corn coleoptiles (*Zea mays* L., cv. WF9 × Bear 38). Preparation of the membrane particles and details of the centrifugation-binding assay were carried out as described previously (Gardner and Sanborn 1989; Sussman and Gardner 1980). Binding experiments were carried out in triplicate, and representative experiments are shown in Tables 1–6. "Specific" binding in these experiments refers to the binding of 10^{-9} M [^{3}H]NPA that is abolished by 10^{-7} or 10^{-5} M nonradio-

Table 1. *Para*-substituted isoquinolinediones: Comparison of in vivo and in vitro activities.

Compound	Whole plant phytotoxicity[a] (pre-/postemergence)	Polar transport[b] (10^{-5} M) (% of control)	Specific binding % of NPA activity[c]	
			10^{-7}	10^{-5}
1 (CEPIQ) 	41/32	2	0	4
2 	27/14	37	56	81
3 	38/6	38	17	18
4 	34/24	16	8	0

[a] Phytotoxicity was evaluated after either pre- or postemergence applications on a numeric scale from 0 to 9, where 0 indicates no visible effect and 9 indicates complete kill, for each of six species. A maximum value of 54 would indicate complete kill of all six species.
[b] Specific (net) polar auxin transport is the amount of radioactivity in the basipetal receiver block minus the amount in the receiver block of the acropetal control. Donor blocks contained 10^{-7} M [^{3}H]indoleacetic acid.
[c] Specific binding is the binding of 10^{-9} M [^{3}H]NPA in corn microsomal membranes that is abolished by 10^{-5} or 10^{-7} M of the competing substance. Values are expressed relative to the specific binding displaced by 10^{-5} or 10^{-7} M NPA. In this experiment the maximum specific binding (±SE), corresponding to 100%, was 5273 ± 88 dpm.

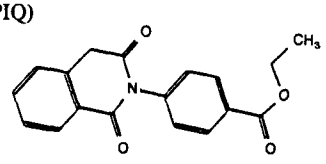
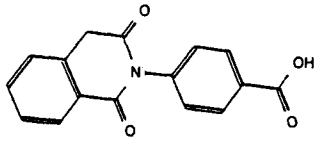
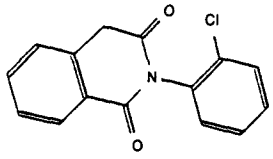
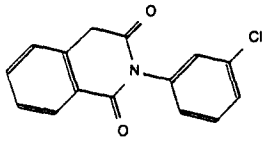
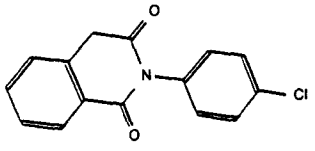
active NPA or a competing substance. Values are expressed relative to the specific binding displaced by the same concentration of NPA. In the experiment shown in Table 1, for example, the maximum specific binding (i.e., 100%) (±SE) was 5273 ± 88 dpm. Thus, the experimental variability within an experiment was about 2%. However, there was quantitative variability from experiment to experiment, and critical comparisons of analogues were therefore made within the same experiment. Other examples of similar data can be found in Gardner and Sanborn (1989).

Auxin transport was directly measured through sections of 5-day-old etiolated corn coleoptiles of the same variety, as described by Hertel and Flory (1968). Primary leaf tissue was removed, the apical 3-mm end of each coleoptile was discarded, and ten 4-mm sections were placed on a receiver block of 1.5% agar containing 0.02 M potassium phosphate buffer, pH 6.4, and the test compound at 10^{-5} M. A donor block in the same buffer, containing 10^{-7} M [^{3}H]indoleacetic acid (IAA), was placed on top of the sections, and basipetal transport (apex to base) was carried out for 90 min. After the transport period, each receiver block was extracted in 10 ml of Beckman Ready-Solv HP scintillation cocktail, and radioactivity was determined by liquid scintillation spectrometry. Nonspecific, primarily diffusional, acropetal transport (base to apex) was determined in a similar way by inverting the coleoptile sections. Each transport test with 10 sections was carried out in duplicate. Specific polar transport was calculated by subtracting from the radioactivity in the basipetal receiver block the amount in the receiver block of the acropetal control.

Whole plant phytotoxicity was measured on six species of weeds after both preemergence and postemergence applications as previously described by Gardner et al. (1985). Phytotoxicity was evaluated on a numeric scale from 0 to 9, where 0 indicates no visible effect and 9 indicates complete kill (no living tissue). In Tables 1–6, as a relative measure of in vivo activity, the scores are summed; therefore, a value of 54 would indicate complete kill of all six species.

The synthesis of the basic 2-aryl-1,3(2H,4H)-isoquinolinediones (homophthalimides) was carried out by reaction of homophthalic anhydride with the appropriate substituted aniline in refluxing xylene or *o*-dichlorobenzene. Metabolic ring-opening of a homophthalimide could result in formation of either a homophthalamic acid or, as discussed below, a phthalamic acid. These were prepared with phenyl substituents corresponding to the compounds in Table 1, along with the appropriate phthalimide for comparison. The synthesis of the homophthalamic acids is depicted in scheme 1. Reaction of the various *para*-

Table 2. Isoquinolinediones: Comparison of in vivo and in vitro activities.

Compound	Whole plant phytotoxicity (pre-/postemergence)	Polar transport (10^{-5} M) (% of control)	Specific binding % of NPA activity	
			10^{-7}	10^{-5}
1 (CEPIQ) 	41/32	14	0	4
5 	9/0	95	0	0
6 	4/18	110	30	18
7 	34/27	9	51	82
2 	27/14	45	56	81

Details as in Table 1.

substituted aniline derivatives with homophthalamic anhydride in refluxing 1,2-dichloroethane led to the target homophthalamic acids in 40–76% yields. In an analogous fashion, treatment of phthalic anhydride with the same aniline derivatives in refluxing 1,2-dichloroethane led to the expected phthalamic acids in 32–70% yields (scheme 2). Compound 16 was purchased from Aldrich Chemical Co. (Milwaukee, WI, USA). Finally, dehydration of the phthalamic acids by fusion led to the corresponding *N*-(*p*-substituted) phenylphthalimides in 53–88% yields (scheme 3).

[5-(n)-^{3}H]IAA was purchased from Research Products International Corp. (Elk Grove, Illinois), and, for these experiments, the original specific activity of 25 Ci/mmol was diluted to 250 mCi/mmol with equimolar nonradioactive IAA.

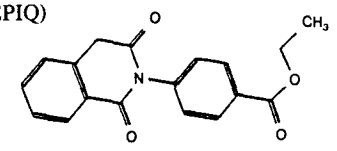
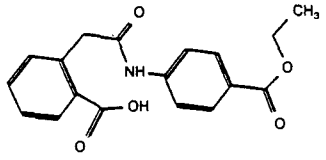
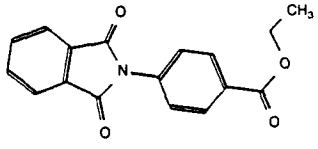
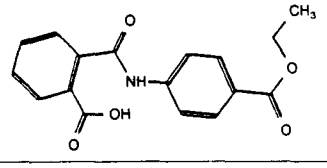
Results and Discussion

A comparison of four analogues with *para*-substitutions on the phenyl ring is shown in Table 1. All four were active in the whole plant screen, but only the *p*-Cl compound (compound 2) showed strong activity in the binding assay. Direct effects on polar auxin transport, however, showed good agreement with herbicidal activity, indicating that the discrepancy was between the binding assay and inhibition of auxin transport rather than between the mechanism and the whole plant.

Since most auxin-transport inhibitors require a free carboxyl group for in vitro activity (Thomson and Leopold 1974), it was thought that the active form of CEPIQ might be its free acid, compound 5. However, this compound (Table 2) was inactive, both in vitro and in the whole plant screen, as well as in the transport test. Since the flavonoid morin had been identified as active (Gardner and Sanborn 1989), it was then concluded that an acidic proton, perhaps on the methylene carbon in the case of CEPIQ, would suffice in place of a carboxyl group.

Another discrepancy occurred with analogues substituted at the *ortho*-position. Such compounds demonstrated weak herbicidal activity, but some

Table 3. *Para*-carboxyethyl isoquinolinedione analogues: Comparison of in vivo and in vitro activities.

Compound	Whole plant phytotoxicity (pre-/postemergence)	Polar transport (10^{-5} M) (% of control)	Specific binding % of NPA activity	
			10^{-7}	10^{-5}
1 (CEPIQ) 	41/32	2	4	16
8 	11/15	110	22	38
9 	0/10	114	32	13
10 	12/11	99	10	26

Details as in Table 1.

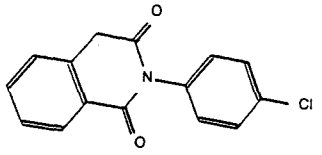
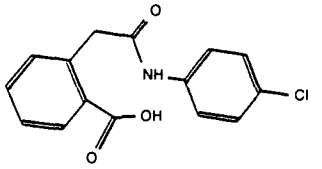
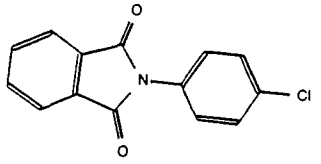
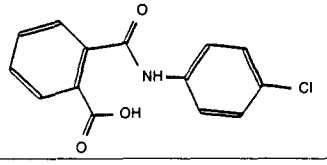
showed significant binding activity. One such example is the *o*-Cl compound (compound 6, Table 2). Again, for these positional isomers, there was a good correlation between herbicidal activity and inhibition of auxin transport.

Thus far in all cases examined, compounds in this class that are highly active on whole plants are good inhibitors of auxin transport, and compounds that are weak as herbicides show little or no effect on auxin transport. It is reasonable, then, to conclude that the mode of action of these isoquinolinedione herbicides is the inhibition of auxin transport. Why then is there an apparent discrepancy between the physiological and biochemical assays, transport and binding? One possible explanation is that compounds of this class express their herbicidal activity by undergoing ring-opening in vivo, yielding products that are more straightforward analogues of NPA with free carboxyl groups. If this explanation is valid, inhibition of auxin transport might be a function of three distinct processes: (1) binding of the parent molecule to the NPA receptor (which is certainly real for some of the halo-substituted compounds), (2) the propensity toward ring-opening (which might be enhanced by substituent patterns quite different from those optimal for receptor binding), and (3) binding of the metabolite to the receptor.

A precedent for metabolic generation of active plant growth regulators is found in the experimental compound DPX-1840 (Beyer et al. 1976). This compound, which is also an inhibitor of auxin transport, undergoes ring-opening in vivo, yielding a product that is more active and has the free carboxyl group typical of compounds with this activity. Unpublished studies (G. Gardner) have shown that DPX-1840 is inactive in the NPA-binding assay, whereas the ring-opened form is very active.

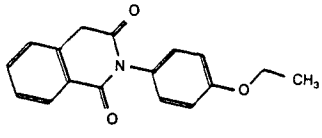
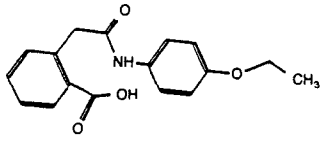
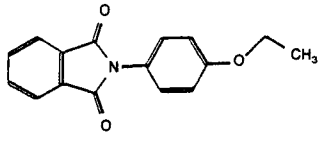
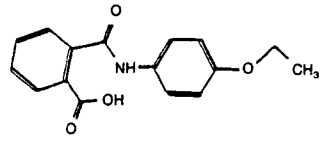
If this explanation is valid for the isoquinolinediones, one would expect that ring-opened forms of molecules such as CEPIQ or compound 4 would be active in vitro, unlike their parents. The compounds selected for further investigation were those from Table 1 that were quite active in vivo but showed discrepancies between the physiological transport activity and the biochemical binding assay. CEPIQ is a striking example of that phenomenon, and it is compared with the related *para*-carboxyethyl derivatives in Table 3. CEPIQ was the only highly active compound in vivo among the four. The homophthalamic acid (compound 8) had very low herbicidal activity, no effect in the auxin transport assay, and

Table 4. *Para*-chloro isoquinolinedione analogues: Comparison of in vivo and in vitro activities.

Compound		Whole plant phytotoxicity (pre-/postemergence)	Polar transport (10^{-5} M) (% of control)	Specific binding % of NPA activity	
				10^{-7}	10^{-5}
2		27/14	41	56	81
11		11/15	85	0	28
12		8/24	102	0	0
13		22/17	78	0	22

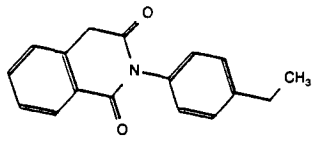
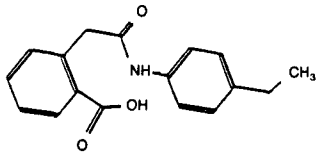
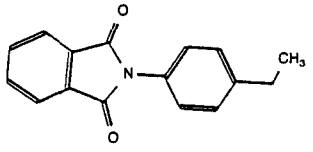
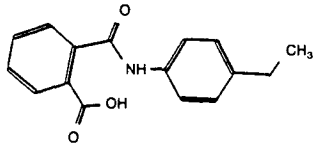
Details as in Table 1.

Table 5. *Para*-ethoxy isoquinolinedione analogues: Comparison of in vivo and in vitro activities.

Compound		Whole plant phytotoxicity (pre-/postemergence)	Polar transport (10^{-5} M) (% of control)	Specific binding % of NPA activity	
				10^{-7}	10^{-5}
3		38/6	62	0(17)	9(18)
14		8/9	104	0	0
15		7/0	110	0	0
16		19/17	70	(31)	(83)

Details as in Table 1. Values in parentheses are from a separate experiment.

Table 6. *Para*-ethyl isoquinolinedione analogues: Comparison of in vivo and in vitro activities.

Compound		Whole plant phytotoxicity (pre-/postemergence)	Polar transport (10^{-5} M) (% of control)	Specific binding % of NPA activity	
				10^{-7}	10^{-5}
4		34/24	38	6	9
17		9/13	88	6	0
18		0/6	105	0	0
19		26/14	71	20	60

Details as in Table 1.

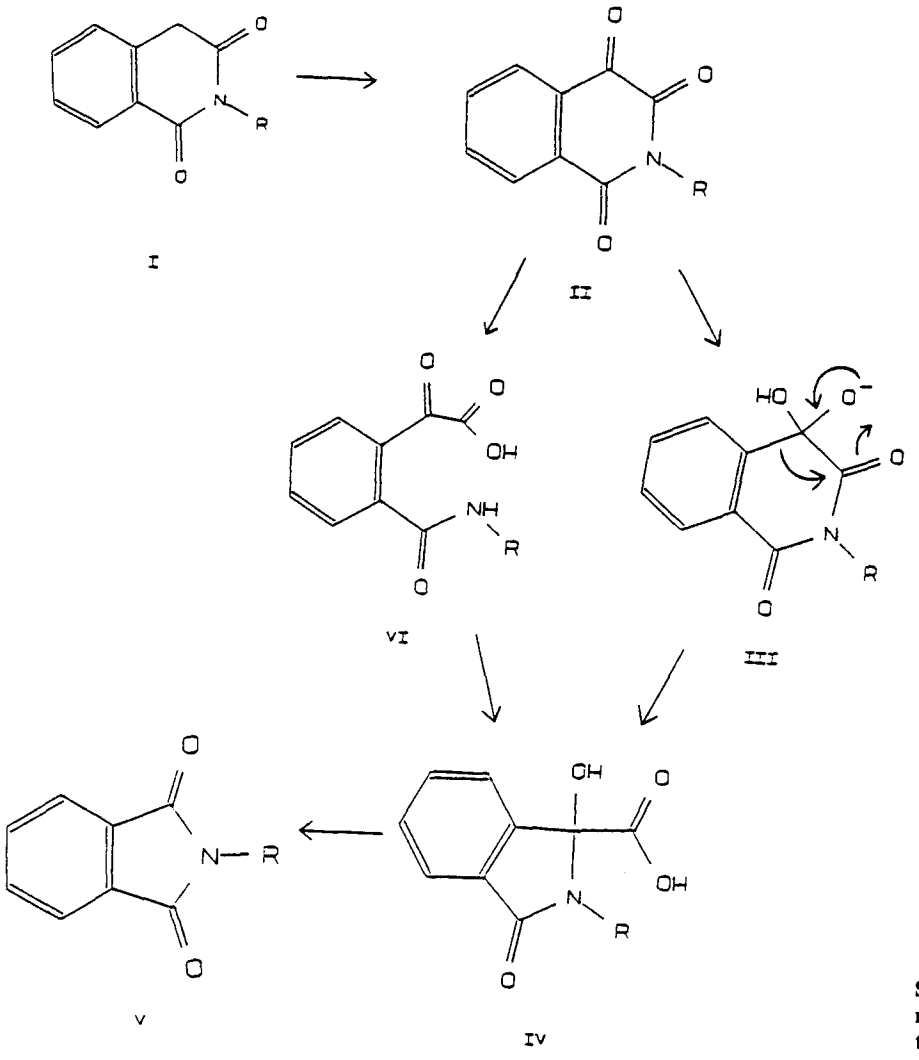
slightly increased binding activity, as did the phthalamic acid (compound 10). Compound 9, the phthalimide, also had little effect in any of the test systems. Since CEPIQ was extremely active in inhibiting auxin transport but showed little or no binding activity, its behavior cannot be accounted for by conversion either to the corresponding phthalamic or homophthalamic acid.

A similar conclusion can be drawn for the *para*-chloro analogues (Table 4). In this case, the homophthalimide compound 2 showed high activity in vitro, as well as in vivo, suggesting that this compound is active in itself. Either ring-opened form is significantly less active than the parent in both in vitro measures.

The structure–activity patterns among the *para*-ethoxy (Table 5) and *para*-ethyl (Table 6) compounds are somewhat different. In both cases, the phthalimides and the homophthalamic acids are relatively inactive across the board. However, the phthalamic acids (compounds 16 and 19) showed moderate inhibition of auxin transport, comparable to that of the corresponding homophthalimides, and relatively high competition at the NPA-binding site. Thus, the results in Tables 5 and 6 support the hypothesis that the homophthalimides act by undergoing ring-opening, yielding more straightforward analogues of NPA.

Tables 4–6 are consistent with the above suggestions that inhibition of auxin transport by the isoquinolinediones might be a function of the three processes mentioned above: (1) binding of the parent, (2) ring-opening, and (3) binding of the metabolite; however, the high activity of CEPIQ still cannot be explained. These latter experiments were undertaken to try to resolve the apparent discrepancy between the auxin-transport and binding assays. Of course, if the compounds are metabolized to something other than the straightforward phthalamic acids, we could not draw valid conclusions based on the present data. Radiosynthesis of CEPIQ was carried out, and preliminary experiments (not shown) indicated that [^{14}C]CEPIQ was rapidly metabolized in corn coleoptiles under conditions of the transport assay; the products have not yet been identified.

Another possible candidate for the metabolite arises from the benzilic acid rearrangement of isoquinolinediones to phthalimides, as described by Smith and Kan (1961) (scheme 4, where R = H). Water could readily add to the phthalimide (V) to form the phthalamic acid. It is conceivable that this chemical oxidation (I → II) could occur enzymati-



Scheme 4. Benzilic acid rearrangement of isoquinolinediones to phthalimides.

cally in vivo. If either the environmental conditions in vivo or the substitution pattern on the phenyl ring stabilizes intermediate IV, this intermediate may be active in itself, resembling the commercial plant growth regulator, morphactin (chlorflurenol). Alternatively, the trione derivative II might be hydrolyzed in vivo to VI, which would then undergo facile ring closure to IV. Similar rearrangements are reported by Petersen and Meitzer (1978). It should be stressed that these suggestions are purely speculative at this time.

Traditional structure–activity principles for auxin-transport inhibitors require that active compounds should possess a carboxylic acid function attached to an aromatic ring which is connected at the *ortho* position to a second aromatic ring (Katekar 1976). Recent evidence presented by Gardner and Sanborn (1989) indicates that the carboxylic acid function can be replaced by an acidic proton. The activity of the isoquinolinediones also supports this revised view. The *para*- and *meta*-chloro analogues (compounds 2 and 7), without free carboxyl groups, are active in competing for NPA-binding sites, as well as in the inhibition of auxin transport. The activity of these compounds, along with observations on flavonoids such as morin (Gardner and Sanborn 1989) and quercetin (Jacobs and Rubery 1988), supports the simpler view that an acidic function is oriented in a precise spatial relationship to an aromatic/hydrophobic ring system (Gardner and Sanborn 1989). However, prediction of in vivo activity is more complex. Within the isoquinolinedione class, three types of structures have been identified: those that are intrinsically active in themselves (compounds 2 and 7); those that might be converted in vivo to traditional, NPA-like analogues (compounds 3 and 4); and those, like CEPIQ, that are not active in vitro and whose met-

abolic fate is unknown. Hopefully, studies with radiolabeled CEPIQ will help to clarify the stereochemical requirements for inhibition of auxin transport.

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