Oxidative Metabolism of Aldrin by Subcellular Root Fractions of Several Plant Species

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Three bean species and three pea varieties demonstrated an *in vitro* aldrin epoxidase activity in root extracts. Although much less efficient, aldrin epoxidase activity also was detected in root extracts of two corn varieties. The epoxidase activity was greatly enhanced by the addition of polyvinylpyrrolidone (Polyclar AT) during homogenization of the root tissue; however, the amount required for optimum results varied with each plant. In all cases, the microsomal fraction was considerably more active as a source of aldrin epoxidase than the 105,000 × *g* solubles. The addition of NADPH generally stimulated the epoxidation of aldrin. Broad bean root extracts were very effective inhibitors of aldrin epoxidation when added to active preparations from peas or Dwarf bean roots. The inhibitory activity was located in the 105,000 × *g* soluble fraction. No metabolism of heptachlor was detected in any plant preparation.

The *in vitro* oxidation of aldrin by enzymes isolated from subcellular fractions of pea and bean plants, principally from the root tissues, has been recently reported (Lichtenstein and Corbett, 1969; Yu *et al.*, 1971). Lichtenstein and Corbett presented a brief review of the literature on aldrin epoxidation, and Yu *et al.* listed the advantages of an *in vitro* system for the investigation of plant pesticide metabolism studies. These reports have provided the background and justification for continued research in this area. However, while attempting to use the foregoing works to establish a procedure for studying the *in vitro* metabolism of certain carbamate insecticides, certain differences in the results of the two reports were noted. Before continuing with the original objective, an investigation designed to resolve or explain these differences was undertaken.

Lichtenstein and Corbett (1969) concluded that the epoxidase activity of pea roots was located primarily in the 105,000 × *g*/2 hr soluble fraction and that the epoxidase did not require added NAD, NADP, or NADPH for activity. Based on these results and the observations that the pea root enzyme system did not epoxidize endrin or heptachlor, the authors concluded that the epoxidase systems in plants were different from those described in animal tissues. The conclusions of these authors were largely supported by studies using a less well-defined enzyme system consisting of cell-free pea root preparations (Oloffs, 1970).

More recently, Yu *et al.* (1971), working with bean and pea root extracts, concluded that the epoxidase enzyme systems in plants were similar to those of mixed function oxidases described from animal tissues. A definite utilization of added NADPH by the bean root epoxidase system was demonstrated. More significantly, the aldrin epoxidase activity was attributed predominantly to the 1 hr 105,000 × *g* pellet (microsome fraction). This was in contrast to the results reported by Lichtenstein and Corbett (1969) for pea roots.

A maximum conversion of 12.4% (using 20 μg of aldrin per incubation) was reported by Yu *et al.* (1971) using bean root extracts, whereas about 1.0% (using 400 μg of aldrin per incubation) conversion of aldrin to dieldrin was reported for pea root extracts (Lichtenstein and Corbett, 1969). Yu *et al.* (1971) found pea root extracts (Freezer variety) to be only half as active as bean (Dwarf Horticulture variety) root extracts. Yu *et al.* (1971) were unable to demonstrate aldrin epoxidase activity in corn root extracts.

In this current work the procedures of Lichtenstein and Corbett (1969) and those of Yu *et al.* (1971) were followed as closely as possible in an attempt to define those techniques which would yield maximum conversion of aldrin to dieldrin. Although it was possible that other metabolites were formed by the plant enzyme systems (Yu *et al.*, 1971), no attempt was made to quantitate or identify products other than dieldrin. In addition, the optimum conditions for enzyme activity were again evaluated and several other plant species screened for heptachlor and aldrin epoxidase activity.

MATERIALS AND METHODS

Chemicals. All insecticides used were analytical grade samples. Aldrin, dieldrin, heptachlor, and heptachlor epoxide were purchased from Unilab Research Corp., Berkeley, Calif. The purity of these compounds was confirmed by the absence of extraneous peaks in gas chromatographs of standards or fortified tissue controls. NADPH was purchased from Sigma Chemical Co., St. Louis, Mo. Polyclar AT powder was supplied gratis from GAF Corp., New York, N.Y. All other chemicals used in the study were analytical grade and pesticide quality solvents.

Plant Materials. Seeds of beans (*Phaseolus vulgaris* L., Dwarf Horticulture variety; *Vicia faba* L., Broad Bean Long Pod variety; and *Phaseolus aureus* Roxb., Mung Bean), peas (*Pisum sativum* L., Alaska Wilt Resistant; and two freezer peas, Miragreen and Little Marvel) and corn (*Zea mays* L., Golden Bantam and PAG-SX29 varieties) were surface sterilized using a 0.5% aqueous solution of sodium hypochlorite for 10 min. After washing several times with water, they were planted in vermiculite flats in the greenhouse. All plant materials used in the study were 2 to 3 weeks old. Roots were cleaned with running tap water and rinsed well with ice-cold distilled water and dried with paper towels. The possibility of microbial enzyme activity was ruled out, since previous reports (Lichtenstein and Corbett, 1969; Yu *et al.*, 1971) indicated no difference between aldrin epoxidase activities of plants, whether or not grown under asceptic conditions.

Tissue Preparation. Ten to 15 g of tissue were homogenized in 2 vol of 0.1 *M* sodium phosphate buffer, pH 6.5, in a Virtis 45 homogenizer operated at medium speed for 2 min. When Polyclar AT was used, the required amount was added to the homogenizing flask and, in some cases, it was necessary to add additional buffer to liquefy thick slurries. The

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homogenate was squeezed through four layers of cheesecloth and the filtrate was referred to as the crude homogenate. The crude homogenate was centrifuged at 22,000 × *g* for 25 min in a Beckman L2-B62 model ultracentrifuge at 4°C. The resultant supernatant, referred to as 22,000 × *g* supernatant, was recentrifuged at 105,000 × *g* for 2 hr. The pellet was resuspended in an original amount of buffer. The supernatant of 105,000 × *g* fraction was termed the 105,000 × *g* solubles, while the resuspended pellet was the microsomal fraction. The above preparations were kept cold at all times, and were used immediately after preparation.

Incubation Procedure. The standard 5-ml incubation mixture consisted of 1-g equivalents of plant tissue preparations, sodium phosphate buffer (0.1 *M*, 6.5 pH), 2 μmol of NADPH, and 400 μg of aldrin dissolved in 2 μl of methyl cellosolve. The reaction was initiated by adding the substrate with a microsyringe. Incubations were carried out in 25-ml Erlenmeyer flasks at 37°C for 4 hr in a metabolic shaker and in an atmosphere of air. Enzyme preparations incubated without substrate and boiled preparations incubated with substrate were used as controls. All incubations were carried out in duplicate.

Extraction and Analysis. The reactions were stopped by adding 4.0 ml of a 2:3 mixture of 2-propanol:hexane. The reaction mixtures were extracted in 15-ml graduated glass-stoppered centrifuge tubes using hexane (Yu *et al.*, 1971). Two milliliters of the 2-propanol:hexane mixture was used to rinse the incubation flasks and the reaction mixture was extracted a second time with hexane. The pooled extracts were adjusted to a final volume of 10 ml and dried with anhydrous sodium sulfate. The extracts were analyzed using a Varian Aerograph model 1700 gas chromatograph equipped with tritium electron capture detector. Standard pyrex glass columns of $^1/_8$-in. × 6-ft, packed either with 10% DC-200 or equal parts of a mixture of 4% SE-30 plus 6% QF-1 on Anachrom ABS (80/90 mesh) were used. The operating conditions were: injector port, 205°C; column, 195°C; detector, 220°C; and nitrogen carrier gas 42 ml per min. Recoveries above 95% were obtained using the above extraction procedure.

RESULTS

Substrate and Tissue Level, Temperature, Buffer, and NADPH Requirements. Conversion of aldrin to dieldrin was studied with respect to the substrate level using Dwarf bean root crude homogenate (Figure 1). There was an increase in dieldrin production even when 3200 μg of aldrin was used per incubation. However, the rate of production declined sharply when the amount of aldrin exceeded 400 μg. Since a larger net amount of dieldrin was produced by using 400 μg of substrate, this level was used routinely in all experiments.

Table I shows the relationship between the amount of tissue equivalents and the net dieldrin production using Alaska pea root extract as the enzyme source. Maximum dieldrin was produced when 0.75 g of tissue equivalents were used. Addition of higher levels of the enzyme preparation resulted in a decline in dieldrin formation. At very low levels of enzyme preparation (less than 0.25-g equivalents) adding NADPH inhibited aldrin epoxidase, but at higher levels NADPH enhanced the activity. Similar action was noticed by Yu *et al.* (1971) working with Dwarf bean root extracts where, at less than 0.52 g tissue equivalents, the addition of NADPH was inhibitory. A reversal of the situation occurred when higher enzyme levels were used. Because of these effects, 1 g equivalent of plant tissue was used with 2 μmol of NADPH for standard incubations. In addition to the two enzyme sources stated above, stimulation of dieldrin production by added NADPH was noted with all other plants used in this study.

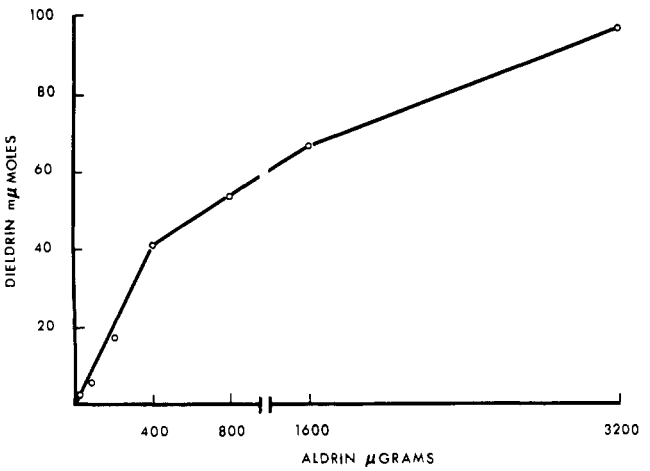

Figure 1. Effect of substrate concentration on aldrin epoxidation by root extracts of beans (Dwarf Horticulture) prepared with 0.25 g of Polyclar AT per gram of tissue

Table I. Effect of Tissue Level and NADPH on Aldrin Epoxidase Activity of Alaska Pea Root Crude Homogenate

Tissue, g	mμmol of dieldrin formed	
	no NADPH	NADPH[a]
0.0	0.0	0.0
0.10	26.83	21.32
0.25	35.19	35.85
0.50	37.87	45.37
0.75	46.52	52.08
1.00	36.04	48.72
1.50	36.04	45.62
2.00	31.00	28.90

[a] 2 μmol of NADPH per incubation.

When the reactions were incubated at 37°C, aldrin epoxidation increased over those incubated at 25°C from 45 to 63%, depending on the enzyme preparation and the plant source. Hence, all standard incubations were carried out at 37°C.

Sodium phosphate buffer (0.1 *M*, 6.5 pH) produced the maximum bean and pea root aldrin epoxidase activity. Tris-HCl (pH 7.2 to 8.5), Tris-maleate (pH 5.5 to 8.5), glycine–NaOH (pH 8.6 to 10.6), and ammediol (pH 8.0 to 10.0) were all inferior or no better than the sodium phosphate buffer.

To find the optimum length of incubation, enzyme preparations were incubated from 0.5 to 20 hr. In boiled-enzyme controls, a maximum of 1.21 mμmol of dieldrin was formed after 4 hr and 2.4 mμmol after 20 hr. The data were corrected for these amounts. The rate of dieldrin formation declined drastically after 4 hr for Alaska peas and after 6 hr for Dwarf bean root extracts (Figure 2). However, dieldrin production continued at a low rate throughout 20 hr of incubation. All standard incubations were carried out for 4 hr.

Effect of Polyclar AT. Extraction and demonstration of a particular enzyme activity in plant tissues are often complicated by the presence of naturally occurring phenolic compounds (Bendall and Gregory, 1963). These authors suggested that in the intact plant the enzymes are presumably separated from these compounds, but as the plant tissue is

Table II. Effect of Polyclar AT in the Extraction Medium on the Aldrin Epoxidase Activity of Various Crude Root Extracts[a]

Plant	mμmol of dieldrin·Polyclar AT in g/g tissue					
	0	0.05	0.1	0.25	0.5	1.0
Peas (Alaska Wilt Resistant)	27.86	31.13	34.79	40.29	20.14	12.81
Beans (Dwarf Horticulture)	20.19	42.40	46.23	49.46	52.49	62.59
Broad Beans (Long Pod)	3.68	4.89	6.97	9.04	10.17	8.49

[a] Enzyme preparation, incubation, and extraction procedures as stated in the text.

Table III. Conversion of Aldrin to Dieldrin (in mμmol) by Various Plant Root Extracts

Fraction	Bean Dwarf Horticulture	Peas			Broad Bean Long Pod	Mung Bean	Corn Golden Bantam
		Alaska Wilt Resistant	Little Marvel	Miragreen			
Crude homogenate[a]	48.29	48.84	31.49	28.12	12.74	5.20	3.72
Crude homogenate[b]	20.19	27.86	23.62	11.48	3.68	2.38	3.28
22,000 × *g* pellet[a]	35.35	38.46	19.69	24.07	12.49	8.85	10.83
22,000 × *g* pellet[b]	16.92	20.28	9.29	13.24	3.28	3.86	4.78
22,000 × *g* soluble[a]	28.39	24.12	18.96	27.01	10.25	6.23	5.14
22,000 × *g* soluble[b]	11.23	15.74	10.41	15.47	2.79	3.09	2.89

[a] Enzyme extracts prepared with 0.25 g of Polyclar AT/g of tissue. [b] Enzyme extracts were prepared without Polyclar AT.

disintegrated, oxidative reactions ensue between the enzymes and the phenolic materials. The products of these polyphenol oxidation reactions, quinones, and condensed products with tanning properties react with enzyme proteins. The resulting complex may be inactive or active, but with modified properties (Bendall and Gregory, 1963). To alleviate such interfering products, Jones and Hulme (1961) used polyvinylpyrrolidone successfully in isolating active mitochondria from plants. Polyclar AT (GAF Corp., New York, N.Y.) is a water-insoluble polyvinylpyrrolidone with a strong affinity for vegetable tannins and binds them irreversibly (Gustavson, 1966). Following the publication of Jones and Hulme (1961), polyvinylpyrrolidone was quite successfully used by several investigators to demonstrate the presence of various enzyme activities in plant tissues (Hulme *et al.*, 1964; Jones *et al.*, 1965; Kleinhofs *et al.*, 1967; Frear *et al.*, 1969).

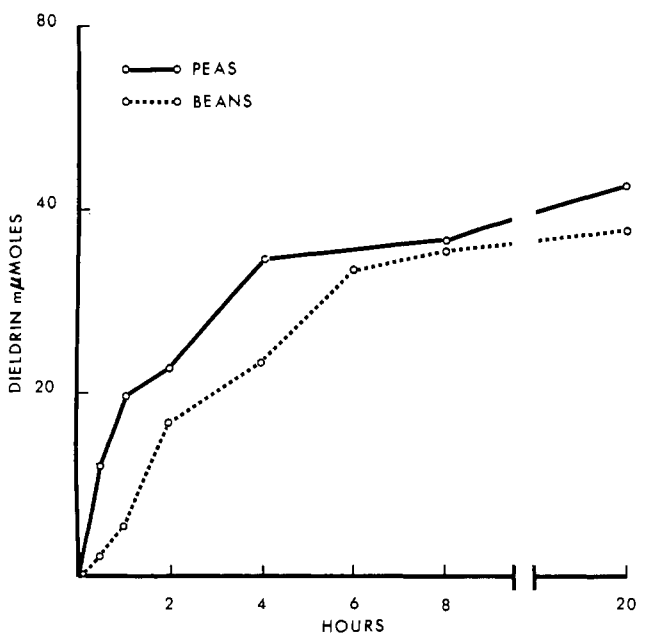

Figure 2. Effect of incubation time on aldrin epoxidation by root extracts of peas (Alaska Wilt Resistant) and beans (Dwarf Horticulture) prepared with 0.25 g of Polyclar AT per gram of tissue

Data presented in Table II show the effect of the addition of Polyclar AT to the extraction medium on the aldrin epoxidation activity of crude root extracts of peas and beans. As little as 0.05 g of Polyclar AT per gram of tissue increased aldrin epoxidase activity in all three plants. The increase was most dramatic in the case of Dwarf bean root extract, showing a twofold enhancement in activity. The amount required for maximum activity varied with each species. The activity increased threefold in Dwarf bean root extract at a level of 1 g of Polyclar AT per gram of tissue. Amounts exceeding 1 g of Polyclar AT per gram of tissue were not used because of the formation of a thick slurry. Optimum level of Polyclar AT for Alaska peas was 0.25 g per gram of tissue, which increased the activity by 45%, whereas 0.5 g per gram of tissue produced a 176% increase in Broad bean root extracts. Amounts exceeding these levels were inhibitory in both cases. Although optimal levels were not determined for all plants, similar increased responses were observed in the other four plants used in the study (Table III). The increased activity was noted in root and/or shoot homogenates as well as with all fractions of differential centrifugation.

Activity in Beans. Dwarf beans were selected for preliminary work based on the report of aldrin epoxidase activity in root extracts (Yu *et al.*, 1971). In general, the present observations essentially confirm the salient features of their report. The 105,000 × *g* soluble fraction was less active than the 105,000 × *g* pellet in all plants tested, irrespective of the use of Polyclar AT (Table IV). A definite response to added NADPH was observed in all root fractions. Addition of Polyclar AT or use of a much higher level of substrate neither altered the pattern of epoxidase activity nor was the total epoxidase activity in Dwarf bean root homogenates improved by addition of calcium chloride and bovine serum albumin. Yu *et al.* (1971) reported these latter compounds enhanced the enzyme activity.

Two additional bean plants were compared for aldrin epoxidase activity (Table III). Broad bean root extracts were

from one-half to one-quarter as active as the corresponding fractions of Dwarf bean roots. The enzyme preparations from these roots darkened within 2 hr after extraction. This darkening was rapidly accelerated upon incubation. Mung beans were much less active than either Dwarf or Broad beans, although they belong to the same genus (*Phaseolus*) as Dwarf beans.

Activity in Peas. Alaska pea roots were the most active of the three varieties of peas tested and were comparable to aldrin epoxidase activity noted in Dwarf beans (Table III). Little Marvel and Miragreen peas (freezer varieties) exhibited considerably less activity. However, their enzyme activities were improved by inclusion of Polyclar AT in the extraction medium. Because improvement in the activity varied with each plant species, a valid comparison of aldrin epoxidase activity would necessitate that the level of Polyclar AT be optimized for each plant.

Activity in Corn. Yu *et al.* (1971) failed to detect aldrin epoxidase activity in corn (Golden Bantam) root extracts. Currently, aldrin epoxidase was detected in corn by using a much higher level of substrate in incubations with corn root extracts (Table III). Although the enzyme in corn roots is an inefficient system, the activity was demonstrated in repeated experiments in both Golden Bantam and PAS-SX29 varieties. The presence of epoxidase activity was also supported by limited *in vivo* experiments using corn seedlings.

Inhibition of Aldrin Epoxidase Activity by Broad Bean Root Extracts. As stated earlier, Broad bean root extracts were unique among the plants used in this study in that the preparations darkened in a short time. These preparations also were very inefficient in aldrin epoxidation (Tables II and III). Darkening of plant preparations has been attributed to the intense level of polyphenol oxidase activity (Jones *et al.*, 1965), products of which interfere with other enzymatic reactions. When crude root extracts of Broad bean (0.05 g equivalents) were added to the reaction mixture of two other plants, it inhibited the aldrin epoxidation by Alaska peas or Dwarf bean enzyme systems. Increasing the Broad bean extract to 0.25 g equivalents resulted in little increase in inhibition of epoxidase activity.

Assay of several centrifugal fractions of Broad bean root extracts showed that the inhibitory activity was in the 22,000 $\times$ *g* supernatant fraction (Table V). The 22,000 $\times$ *g* pellet had little inhibitory activity, and in combination with the 22,000 $\times$ *g* pellet fraction of Dwarf bean roots caused a slight enhancement of enzyme activity. Further centrifugation of the 22,000 $\times$ *g* supernatant fraction at 105,000 $\times$ *g* (2 hr) and examination of these fractions indicated that most of the inhibitory activity was present in the soluble fraction. Also, the 105,000 $\times$ *g* soluble fraction was more inhibitory than the 22,000 $\times$ *g* supernatant fraction. This may have resulted from further accumulation of quinone-like products during the time required for the preparation of the fraction. Ammonium sulfate fractionation of the solubles revealed that the inhibition was not associated with any of the protein fractions.

Heptachlor. All the above plant shoot and root extracts were incubated with 20 or 400 μg of heptachlor with or without added NADPH. No heptachlor epoxide was detected in any of these reactions. Addition of Polyclar AT during homogenization and varying the incubations from 2 to 8 hr produced no heptachlor epoxide.

Table IV. Aldrin Epoxidase Activity of High Centrifugation Fractions of Peas (Alaska Wilt Resistant), Beans (Dwarf Horticulture), and Broad Bean (Long Pod) Root Extracts[a]

Fractions[b]	mμmol of dieldrin		
	Peas	Beans	Broad Beans
105,000 $\times$ *g* pellet	10.73	12.10	3.05
105,000 $\times$ *g* solubles	3.39	4.12	1.03

[a] Root extracts prepared with 0.25 g of Polyclar AT per gram of tissue. All other conditions were as stated in text. [b] 105,000 $\times$ *g* fractions were obtained from 22,000 $\times$ *g* supernatants.

Table V. Inhibition of Aldrin Epoxidation in Peas (Alaska Wilt Resistant) and Beans (Dwarf Horticulture) Root Extracts by Various Fractions of Broad Bean (Long Pod) Root Extracts

Root fraction	Broad Bean fraction, 0.05 g tissue equivalents	% Inhibition
Alaska Pea		
Crude homogenate	Crude Homogenate	68.8
Crude homogenate	22,000 $\times$ *g* pellet	4.0
Crude homogenate	22,000 $\times$ *g* solubles	71.3
22,000 $\times$ *g* pellet	Crude Homogenate	82.5
22,000 $\times$ *g* pellet	22,000 $\times$ *g* pellet	39.7
22,000 $\times$ *g* pellet	22,000 $\times$ *g* solubles	77.3
22,000 $\times$ *g* solubles	Crude Homogenate	72.3
22,000 $\times$ *g* solubles	22,000 $\times$ *g* pellet	14.8
22,000 $\times$ *g* solubles	22,000 $\times$ *g* solubles	73.9
Dwarf Bean		
Crude homogenate	Crude Homogenate	68.0
Crude homogenate	22,000 $\times$ *g* pellet	+15.5[a]
Crude homogenate	22,000 $\times$ *g* pellet	73.2
22,000 $\times$ *g* pellet	Crude Homogenate	80.8
22,000 $\times$ *g* pellet	22,000 $\times$ *g* pellet	+6.9[a]
22,000 $\times$ *g* pellet	22,000 $\times$ *g* solubles	81.6
22,000 $\times$ *g* solubles	Crude Homogenate	70.6
22,000 $\times$ *g* solubles	22,000 $\times$ *g* pellet	+8.1[a]
22,000 $\times$ *g* solubles	22,000 $\times$ *g* solubles	79.6

[a] These reactions increased dieldrin formation when indicated Broad bean root extracts were added.

DISCUSSION

In agreement with the work of Yu *et al.* (1971), but in contrast to the report of Lichtenstein and Corbett (1969), the 105,000 $\times$ *g* pellet was shown to be more active than the 105,000 $\times$ *g* soluble fraction in all plants tested. Addition of NADPH stimulated aldrin epoxidase activity, as was observed by Yu *et al.* (1971). However, the actual requirement of NADPH remains unclear, since it was observed that the response to added NADPH was dependent on the amount of tissue used and the age and physiological condition of the plants.

Generally, the levels of dieldrin production were similar to those reported for Dwarf bean root extracts (Yu *et al.*, 1971) and for Alaska pea root extracts (Lichtenstein and Corbett, 1969).

The level of dieldrin production reported by Yu *et al.* (1971) in peas was less than half of that produced by Dwarf beans. However, data presented in Table III indicate that aldrin epoxidase activity in Alaska peas was comparable to that in Dwarf beans. There was a marked difference in aldrin epoxidase activity in the three pea varieties listed. The difference in aldrin epoxidase activities in peas reported by Yu *et al.* (1971) and that reported by Lichtenstein and Corbett (1969) could be due to the difference in the varieties used in these two studies. This emphasizes the need for investigators to fully identify the plant material used in their study.

The use of Polyclar AT during homogenization of the plant tissue increased the aldrin epoxidase activity. Optimization of Polyclar AT should be established for individual plants,

since preparations vary in response to added Polyclar AT, depending on the plant species. Comparison of aldrin epoxidase activity of various plants used in this study can be best made by considering the preparations without added Polyclar AT, since optimization for all plants was not conducted. Addition of calcium chloride and bovine serum albumin to incubations, along with optimum levels of Polyclar AT, did not enhance the epoxidase activity above that brought about by use of Polyclar AT alone.

The Broad bean root extracts, which were very inefficient in aldrin epoxidation and turn dark upon incubation or standing, were tested for inhibitory activity. Since darkening of plant preparations is thought to be an indication of intense polyphenol oxidase activity (Jones *et al.*, 1965), it was possible that the low aldrin epoxidase activity was caused by the presence of quinolic products resulting from polyphenol oxidase activity. A very effective inhibitory activity was located in the soluble fraction of the Broad bean root extracts. This activity was not associated with the protein precipitates. Characterization and identification of this inhibitor could provide a useful tool for the elucidation of the fate of aldrin and other pesticides in plants.

Use of higher substrate levels was beneficial in this study and permitted detection of aldrin epoxidase activity in corn roots previously reported to be devoid of this activity (Yu *et al.*, 1971). Incubations for longer than 2 hr also proved advantageous in obtaining higher levels of dieldrin. However, further research will be required for the optimization of the total incubation parameters and for the most efficient procedures for extracting active enzymes from plant material.

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Metabolism of 4-Chloro-2-butynyl-3-chlorocarbanilate by Soybean Plants

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Root-treated soybean plants absorb, translocate, and metabolize 4-chloro-2-butynyl-3-chlorocarbanilate-^{14}C (barban-^{14}C). Water-soluble products and insoluble residues are rapidly formed in the roots. Only water-soluble residues are found in the shoots after 3 days posttreatment. Time-course experiments indicated a possible precursor product relationship between barban, soluble products, and insoluble residues. Water-soluble metabolites are not translocated once they are formed in the shoot tissues. The radiocarbon distribution in soybean plants root-treated with phenyl-^{14}C, carbonyl-^{14}C, and butynyl(C-1)-^{14}C-labeled barban were identical in all comparative experiments. These studies indicate that the barban molecule was not cleaved in soybean. Barban-phenyl-^{14}C was root fed to soybean plants for 4 days and the polar metabolites were isolated. Caustic hydrolysis of polar metabolites from either root or shoot yielded 3-chloroaniline-^{14}C. The recovery of 3-chloroaniline-^{14}C indicated that barban-^{14}C metabolites are formed by the alteration of the 4-chloro-2-butynyl side chain and not by hydroxylation of the aromatic nucleus, as was found for isopropyl-3-chlorocarbanilate in soybean.

One of the most effective herbicides used in the control of wild oat (*Avena fatua*) has been 4-chloro-2-butynyl-3-chlorocarbanilate (barban). Though this herbicide has been widely used in agriculture, its metabolism by plants is not well understood. Foliar absorption of barban in the various plant species has been reported by Foy (1961), Riden and Hopkins (1962), and Crafts (1964). Foliar translocation both acropetally and basipetally has been confused by conflicting reports. It appears that one could expect only a small percentage of barban to translocate after leaf treatment. Root absorption of barban has been reported (Crafts, 1964; Jacobsohn, 1970) in several plant species. These studies have demonstrated limited acropetal translocation of barban.

Riden and Hopkins (1961, 1962) demonstrated the conversion of barban to a polar metabolite (Compound X), which was shown to be a nonproteinaceous, dialyzable material which yielded 3-chloroaniline upon caustic hydrolysis. The conversion of barban to aniline-containing polar metabolites occurred in 13 different plant species, including grasses and broad leaf plants. The appearance and disappearance of the polar metabolite (Compound X) was a function of time. Jacobsohn (1970) also demonstrated the conversion of leaf-treated barban to polar metabolites and extended the investigation to root-treated wild oat and barley plants. These plants also converted barban to 3-chloroaniline containing polar metabolites. Riden and Hopkins (1962) showed that there was no free 3-chloroaniline in their barban leaf-treated plants. Further, they were able to isolate

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