Enzymatic Conversion of Aldrin to Dieldrin with Subcellular Components of Pea Plants

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Subcellular fractions from peas were obtained by differential centrifugation after the plants had been grown under both sterile and nonsterile conditions. Enzymatic conversion of aldrin to dieldrin was achieved with different cell fractions. Of the total dieldrin recovered from all cell fractions, 11% was produced by the "nuclei"-pellet (1000 G), 18% by the "mitochondria"-pellet (10,000 G), 17% by the "microsome"-pellet and 54% by the soluble fraction (105,000 G). The enzymatic activity was increased with Cu^{2+} and Fe^{2+} by 123 and 56%, and was inhibited by Mn^{2+}, sesamex, and SKF 525-A by 30, 72, and 80%, respectively. The effects of Cu^{2+} and Fe^{2+} could be nullified by the addition of EDTA to the reaction mixtures. The enzyme(s) in peas appeared different from that described in animal tissues, since: It is specific for aldrin and did not epoxidize the endo-endo isomer of aldrin (isodrin) or heptachlor; cofactors, such as NAD, NADP, and NADPH did not affect the rate of epoxidation of aldrin; and most of the enzymatic activity was found in the soluble fraction and not in the "microsome"-pellet.

The epoxidation of chlorinated hydrocarbon insecticides in the animal body was first demonstrated by Davidow and Radomski (1953) for heptachlor and by Bann *et al.* (1956) for aldrin. Nine years later, Nakatsugawa *et al.* (1965) reported that microsome fractions from rabbit liver contained enzyme systems that converted aldrin, isodrin, and heptachlor into their corresponding epoxides. The enzymes that catalyzed this reaction required $NADPH_2$ and oxygen. Conversely, SKF 525-A, piperonylbutoxide, parathion, and γ-BHC inhibited the epoxidation of aldrin and heptachlor. They also found epoxidase activity in rat liver microsomes and insect homogenates. Lewis *et al.* (1967) reported the epoxidation of aldrin with microsomal preparations from houseflies and pig liver, and that "inhibitors of lipid peroxidation such as EDTA, Mn^{2+}, Co^{2+}, α, α-dipyridyl, and BHT (butylated hydroxytoluene) stimulated dieldrin formation." Sun and Johnson (1960) demonstrated that the epoxidation of aldrin in houseflies was inhibited by sesamex and suggested that sesamex inhibits biological oxidations.

The conversion of aldrin to dieldrin in soils was first reported by Edwards *et al.* (1957) and later by Gannon and Bigger (1958). Investigations by Lichtenstein and Schulz (1960) showed that the rate of epoxidation of aldrin and heptachlor in soils was a function of the activity of soil microorganisms. Additional evidence for the biological oxidation of aldrin in soil was presented by Lichtenstein *et al.* (1963) when it was demonstrated that the addition of five different methylenedioxyphenyl synergists to soils or to microorganisms isolated from soils, inhibited the epoxidation of aldrin. Sesamex inhibited this reaction more than any of the other compounds tested.

The epoxidation of aldrin and heptachlor by plant tissues was indicated in field experiments (Lichtenstein, 1960; Lichtenstein and Schulz, 1965). They found that different crops grown in the same aldrin or heptachlor-treated soil contained different amounts of either dieldrin or heptachlor epoxide when expressed in per cent of the totally recovered insecticidal residues. In other experiments (Lichtenstein *et al.*, 1967), peas were grown in aldrin-treated quartz sand of minimal biological activity. Although only 1% of the total residue recovered from the sand at harvest time was in the form of dieldrin, this figure amounted to 11% in the pea roots and to 93% in the pea greens. This indicated that aldrin penetrated into the roots as aldrin, where it was partially converted into dieldrin, followed by translocation into the pea greens. Oloffs and Lichtenstein (1969) demonstrated in laboratory experiments that oxygen was required for the epoxidation of aldrin in plant tissues, and that this reaction was inhibited by heat or the addition of enzyme inhibitors such as KCN and 2,4-DNP.

In this paper, investigations are described relative to the conversion of aldrin to dieldrin by subcellular fractions of pea plants, primarily pea roots.

MATERIALS AND METHODS

Chemicals. All the insecticides used were of analytical grade. Aldrin, dieldrin, isodrin (endo-endo isomer of aldrin), and endrin (endo-endo isomer of dieldrin) were obtained from the Shell Chemical Co., heptachlor and heptachlor epoxide from the Velsicol Chemical Corp., sesamex from Shulton, Inc., and SKF 525-A (β-diethylaminoethyl diphenylpropylacetate hydrochloride) from Smith, Kline & French Laboratories. NADP, NADPH,

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and NAD were purchased from the Nutritional Biochemical Corp.

The insecticides were applied in an ethanol solution, NADP, NADPH, and NAD in 0.1*M* sodium phosphate buffer—pH 7.0, and SKF 525-A in water. Sesamex was dissolved in 0.1*M* ethanol and an aqueous solution was prepared prior to the experiments. A sodium-phosphate buffer solution at 0.1*M* and pH 7.0 was used in all the experiments.

Plant Material. Pea seeds (Alaska Wilt Resistant or Onwards) were surface sterilized by soaking them for 10 minutes in a 0.5% aqueous solution of sodium hypochlorite (Sanwal, 1963) to obtain a high germination rate. After washing the seeds for 2 hours in running tap water, they were planted in quartz sand within borosilicate glass dishes. The sand was watered with tap water as necessary while the developing plants were grown in the greenhouse or in the laboratory under GRO-LUX lamps (temperature = 22 ± 2° C., 10 hours of light per day). The plants were used for experimental purposes when the pea greens reached a height of 10 to 12 cm. To obtain etiolated stems and leaves, pea plants were also grown in the absence of light.

Sand particles adhering to the root surface were removed with running tap water. The roots were then washed in glass-distilled water and partially dried with paper towels.

To find if enzymatic activity was due to microorganisms attached to the root surfaces, roots were also grown under aseptic conditions. The seeds were surface sterilized as described, followed by eight successive washings in 150-ml. portions of sterile glass-distilled water. These seeds were then germinated on nutrient agar, containing 4.0 grams of agar, 2.0 grams of glucose, 0.2 gram of NH_4NO_3, 0.2 gram of K_2HPO_4, 0.1 gram of $MgSO_4 \cdot 7H_2O$ and 0.2 gram of yeast extract per liter of H_2O. This medium, when solidified, had such a consistency that seeds did not sink into the medium, yet roots could still penetrate into it. It also indicated any accidental contamination by microorganisms after a few days of incubation at room temperature. Test tubes (20 cm. high, 2.2 cm. i.d.) were filled to a height of 9 cm. with nutrient agar, followed by sterilization in an autoclave. After cooling, three to four of the surface sterilized seeds were placed onto the solidified agar within a transfer chamber. The tubes were again plugged with cotton and kept in the dark at room temperature for seven to 10 days, during which time the roots reached a length of 5 to 7 cm.

Preparation of Subcellular Fractions for Enzyme Activity Tests. All procedures to obtain plant fractions for enzyme activity tests were carried out at 0 to 4° C. Plant material was homogenized with either grinding medium or buffer solution (pH 7.0) in a 1-to-2 ratio. Depending on the amount of plant material, a mortar or a Waring Blendor was used. The grinding medium consisted of 0.3*M* mannitol, 1.0m*M* EDTA, and 0.05% cysteine, pH 7.2, as described by Bonner (1967), except that no BSA was used. The cysteine was added immediately prior to the preparation of the homogenate. The resulting homogenate (I) was squeezed through cheese cloth. Various cell fractions were then obtained by differential centrifugation according to the following

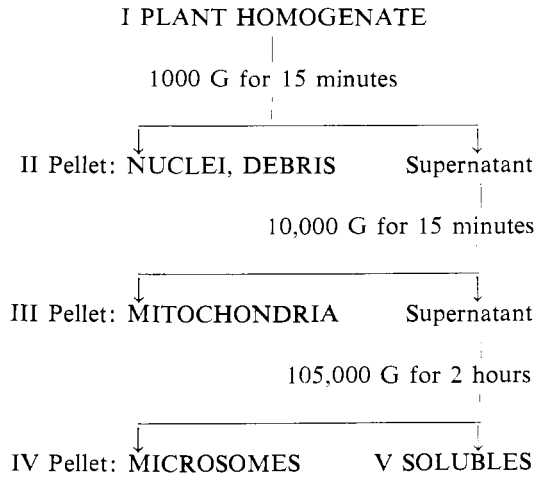

diagram. G-forces refer to the average radius of the centrifuge tubes.

In experiments conducted at the beginning of this investigation, pellets were resuspended in 10 to 15 ml. of grinding medium. However, this medium inhibited, to some extent, the epoxidation of aldrin, primarily because of the presence of EDTA. Therefore, in most of the experiments, plant material was ground, resuspended, and incubated only with 0.1*M* sodium phosphate buffer, pH 7.0. The standard reaction mixtures, consisting of 0.5- to 2.0-ml. aliquots of the various plant fractions (I to V), were diluted to 10 ml. with buffer solution and held in 50-ml. Erlenmeyer flasks to which 400 μg. of insecticide in 20 μl. of ethanol was added prior to incubation. The glass stoppered flasks were then shaken for 2 hours in a water bath of a gyratory shaker at 30° C. and 120 r.p.m.

When experiments were conducted with aseptically grown material, only those roots not contaminated by microorganisms were used for enzyme activity tests. If anaerobic microorganisms had developed, this would have shown up in the lower agar layers, especially along the root surfaces. All the glassware, the buffer solution, and other tools were sterilized before use. The plants were removed from the agar in a transfer chamber; roots were washed in sterile glass-distilled water and processed at 0 to 4° C. as described.

Protein Precipitation and Enzyme Purification. To determine the need for cofactors, a protein precipitate of a root homogenate was used as the enzyme source. For this purpose proteins were precipitated with ammonium sulfate at 0 to 70% saturation. After centrifugation at 10,000 G for 10 minutes, the protein pellet was resuspended in 0.1*M* sodium phosphate buffer (pH 7.0) and dialyzed for 1 hour against 0.01*M* buffer. After additional centrifugation at 10,000 G for 10 minutes, the supernatant was used as the enzyme source.

A second root homogenate was used to obtain heat stable and dialyzable cofactors. For this purpose, one portion of the root homogenate was heated for 10 minutes in boiling water, while the rest (75 ml.) was dialyzed against 250 ml. of 0.01*M* buffer for 18 hours. The solution surrounding the dialysis bag (dialyzate) was then concentrated at 17° C. by means of a flash evaporator to 30 ml. and used for testing.

To achieve some purification of the enzymes or

enzyme systems, fractional precipitations of proteins were performed in two separate experiments. Root homogenates were prepared as described, followed by a protein precipitation with ammonium sulfate at 70% saturation. After centrifugation at 10,000 G for 10 minutes, the protein pellet was resuspended in 150 ml. of buffer solution and dialyzed for 1 hour against 5 liters of 0.01*M* buffer. After an additional centrifugation (10,000 G for 10 minutes) of the dialyzed resuspended pellet, the resulting supernatant was saturated with ammonium sulfate between 0 to 17.5%, 17.5 to 35%, 35 to 52.5%, and 52.5 to 70%. Aliquots of the original root homogenate, the total 0 to 70% protein fraction, and the four separate fractions showing various degrees of saturation were then used for protein determination and incubation with aldrin as described.

Extraction and Analyses. The reaction mixtures were either extracted immediately after incubation or frozen until extraction was possible. In the first three experiments of this study, redistilled hexane was used for extraction. However, recoveries of aldrin and dieldrin were very low, especially in the presence of relatively large amounts of organic material. Only 40 to 50% of the amounts of aldrin or dieldrin, added to a plant homogenate (I) before incubation, were recovered by this procedure. Reaction mixtures were subsequently extracted with a 1-to-1 mixture of redistilled hexane and redistilled acetone. Aldrin or dieldrin added to plant homogenates were recovered to an extent of 90 to 96% after a 4-hour shaking period. Data reported in this paper are based on results obtained with hexane-acetone as the extraction solvents.

The amount of aldrin in the reaction mixture was far above its water solubility (0.01 p.p.m.) The major part of the substrate was, therefore, not in solution and apparently was attached to organic materials in the reaction mixture. This would account for the inability to extract fully the insecticides with hexane only.

After extraction, the acetone was removed from the hexane-acetone-water mixture by washing with water. The hexane fraction was then dried over anhydrous sodium sulfate, adjusted to volume, and analyzed by gas-liquid chromatography. Two instruments were used for this purpose. One was an Aerograph gas chromatograph, Model 204, in which a 1.22-meter glass column (4.0-mm. i.d.) containing 3% polyethylene glycol adipate on Chromosorb W, 100- to 120-mesh was used. A column pressure of 60 p.s.i. of nitrogen gave a flow rate of 50 ml. per minute. The injector temperature was maintained at 195° C. and the detector cell at 200° C. The oven temperature was 185° C. The other instrument was a Jarrell-Ash gas chromatograph, Model 28-700, and the conditions were those as described by Lichtenstein *et al.* (1967).

Thin-layer chromatography was used in three cases for confirmatory tests. Extracts of different reaction mixtures, after analysis by gas-liquid chromatography, were concentrated and spotted on aluminum oxide G coated glass plates. After the chromatograms had been developed with a mixture of heptane and ethyl acetate (9 to 1), they were sprayed with reagents as described by Mitchell (1957), followed by exposure to UV light for 10 minutes. In addition to aldrin (R_f 0.67), dieldrin (R_f 0.45) was detected in all cases.

Protein determinations were performed according to the method as described by Lowry *et al.* (1951), using crystallized bovine serum albumin as a standard.

RESULTS AND DISCUSSION

Evidence for Enzyme Activity. Various experiments were conducted to demonstrate that the conversion of aldrin to dieldrin was indeed an enzyme catalyzed reaction. First, in the absence of plant material, no dieldrin was produced. In three additional tests, aliquots of the various plant cell fractions (I to V) were heated for 10 minutes in boiling water, thus achieving a protein denaturation. No dieldrin was detected in any of the reaction mixtures which had been incubated for 2 hours with boiled plant material. However, dieldrin was produced when aliquots of the nonheated plant cell fractions were used.

Usage of increasing amounts of plant material resulted in a corresponding increase in enzyme activity as measured by dieldrin formation. This was most evident with the soluble fraction (V), up to 5.2 mg. of protein.

To determine the need for cofactors, a protein precipitate of a root homogenate was used as the enzyme source, and a boiled root homogenate or a dialyzate was used for the testing of heat stable and dialyzable cofactors. For this purpose, 1.5- and 3.0-ml. aliquots of either the boiled homogenate or the dialyzate were added to the enzyme source. Experiments with the protein fractions only served as controls. The addition of the boiled homogenate or the concentrated dialyzate to the enzyme preparation increased the production of dieldrin by factors of 1.3 and 1.5, respectively. This indicated that a heat stable substance of relatively low molecular weight was present in the boiled plant homogenate and the dialyzate, which increased the enzymatic conversion of aldrin to dieldrin.

Location of Enzyme Activity. The total amount of dieldrin produced by one specific cell fraction was calculated from the amount of dieldrin that had been produced by the aliquots used for incubation with aldrin. This determined the amount of dieldrin produced by all four cell fractions obtained through differential centrifugation and the per cent distribution of dieldrin within these fractions. Results (Table I) from five different experiments (mean values plus standard deviation) showed that of the total dieldrin recovered, 54%

Table I. Dieldrin Produced from Aldrin by Four Cell Fractions, Obtained through Differential Centrifugation of Pea Root Homogenates

Cell Fraction	Dieldrin in % of Total Amount Recovered from All 4 Cell Fractions[a]
Nuclei (1000 G/15 min.)	11 ± 3
Mitochondria (10,000 G/15 min.)	18 ± 6
Microsomes (105,000 G/2 hours)	17 ± 6
Solubles (105,000 G/2 hours)	54 ± 11

[a] Results of five different experiments: mean value plus standard deviation.

had been produced by the soluble fraction (supernatant at 105,000 G), while the nuclei, mitochondria, and microsome pellets produced similar amounts, totaling 46%. Similar results were obtained with cell fractions prepared from etiolated pea stems and leaves.

In two additional tests, pea root homogenates were centrifuged directly at 105,000 G for 2 hours. The resuspended pellet (nuclei, mitochondria, and microsomes) and the supernatant were then used for incubation with aldrin. Results showed that 54 and 58% of the total dieldrin recovered had been produced by the supernatant. In another test, the supernatant obtained during a 2½-hour centrifugation at 151,000 G produced 52% of the total dieldrin recovered.

Enzyme Preparations from Roots Grown under Aseptic Conditions. All the experiments described so far had been conducted with roots that were not grown under aseptic conditions. It could, therefore, have been possible that the epoxidizing enzyme originated from microorganisms that were attached to the root surfaces. This, however, seemed unlikely since over 50% of the enzymatic activity was located in the soluble fraction (105,000 G).

Additional proof that the enzyme did not originate from root surface microorganisms was obtained when aseptic root homogenates were used as the enzyme source. Aliquots of these homogenates were incubated as described with sterile buffer solution and aldrin in sterilized Erlenmeyer flasks. Results obtained were identical with those obtained with roots that had not been grown under aseptic conditions.

Enzyme Purification. To find if a purification of the enzyme(s) had been achieved, aliquots of a root homogenate and the various protein fractions described above were incubated with aldrin for 2 hours.

The specific activity of each fraction was calculated by determining the amount of dieldrin (micrograms) that was produced in 2 hours by the amount of protein (milligrams) present in 1 ml. of a particular fraction. These specific activities were 1.24 ± 0.07 (root homogenate), 1.13 ± 0.17 (0 to 70%), 1.01 ± 0.40 (0 to 17.5%), 0.55 ± 0.10 (17.5 to 35%), 0.71 ± 0.21 (35 to 52.5%), and 0.56 ± 0.30 (52.5 to 70%). This decrease in specific activity could have been due to an increasing denaturation of the oxidizing enzyme during the various protein precipitation processes. It could also have been possible, however, that necessary cofactors were lost during the different dialyses procedures that were required for the preparation of the various protein fractions.

To test if indeed the protein had been denatured or if a purification of pea root protein had been achieved, a different substrate (shikimic acid) was used, measuring the activity of the enzyme dehydroshikimic reductase by utilizing the same protein fractions from pea roots. This enzyme, which "catalyzes the reduction of dehydroshikimic acid, utilizing the reduced form of triphosphopyridine nucleotide as coenzyme" (Balinsky and Davies, 1961), was chosen since it is simple to assay. The conversion of NADP to NADPH was measured, and specific activities were calculated for all protein fractions. Expressing this activity of the total protein fraction (0 to 70%) as 1.0, specific activities of 0.15 (0 to 17.5%), 0.55 (17.5 to 35%), 1.83 (35 to 52.5%), and 2.44 (52.5 to 70%) were obtained. This increase in the specific activities indicated that the enzyme dehydroshikimic reductase had not been denatured. Assuming that the protein of the enzyme that catalyzes the aldrin epoxidation was also not affected, apparently necessary cofactors were lost during the dialysis procedures.

Cofactor Requirements. The effect of EDTA (ethylene diamine tetraacetate) on the epoxidation of aldrin with plant fractions was studied in several experiments. Initially, aldrin was incubated in a mixture of plant material, grinding medium, and sodium phosphate buffer. When the grinding medium was replaced by buffer solution, 2.0 to 2.5 times more dieldrin was produced. To determine which component in the grinding medium was responsible for this inhibition, enzymes (protein precipitate of pea root homogenates at 0 to 70% ammonium sulfate saturation) and aldrin were incubated with only a buffer solution, with buffer plus grinding medium, with buffer plus 1m*M* EDTA, with buffer plus 0.3*M* mannitol, or with buffer plus 0.05% cysteine. Considering the amount of dieldrin produced in the presence of plant material and buffer only as 1.0, the addition of grinding medium reduced this production by 47 and 58%, of EDTA by 38%, while the addition of mannitol or cysteine had no effect. In experiments described later, addition of EDTA at 5m*M* resulted in a 57 and 65% reduction of enzyme activity. EDTA apparently chelated metals which serve as necessary cofactors for the aldrin-epoxidizing enzyme.

The need for a heat stable cofactor of low molecular weight was indicated in previously described experiments with boiled plant homogenates and dialyzates. In the following tests, an attempt was made to remove cofactors from the plant material through dialyses and replacing them with metal ions. For this purpose, the soluble fraction from pea roots was used in a nondialyzed form as well as after dialyzing for 1.5 hours against 0.01*M* buffer. Protein determinations were made, and the enzyme preparation was incubated with aldrin for 2 hours. Results from duplicated tests showed that the specific activity of the nondialyzed fraction was 0.57 ± 0.05 and of the dialyzed fraction 0.40 ± 0.04. However, the addition of $CuSO_4 \cdot 5\ H_2O$ at 0.5m*M* to the dialyzed plant fraction again increased this production of dieldrin, resulting in a specific activity of 0.51 ± 0.01. The concentration of a necessary cofactor apparently decreased during dialysis, and the addition of cupric sulfate partially restored the original enzyme activity.

Various metal ions were then added to enzyme preparations which were obtained by precipitating the proteins in a pea root homogenate with ammonium sulfate at 0 to 70% saturation. Results of these tests are summarized in Table II. They show that copper and iron ions—known as cofactors of many oxidative enzymes—increased the epoxidation of aldrin. Especially active were Fe^{2+} and Cu^{2+} ions, though cuprous chloride (Cu^{+}) had a similar effect. When EDTA was added at 5m*M* to reaction mixtures containing either ferrous sulfate (Fe^{2+}, 0.5m*M*) or cupric sulfate (Cu^{2+}, 0.5m*M*), the effect of the metal ions was nullified. A 57 and 65% reduction in the dieldrin production had occurred when compared to those reaction mixtures which contained metals but no EDTA. The addition

Table II. Effect of Metals on Epoxidation of Aldrin by Pea Root Enzyme Preparations[a]

Salts	Metal Concentration	
	1m*M*	0.5m*M*
	Dieldrin Recovery in % of Control	
None	100[b]	100[c]
$FeSO_4 \cdot 7\ H_2O$	171 ± 12	156 ± 8
$FeCl_2 \cdot 4\ H_2O$[d]		167 ± 5
$Fe_2(SO_4)_3$	168	130 ± 4
$FeCl_3 \cdot 6\ H_2O$	124 ± 5	
$CuSO_4 \cdot 5\ H_2O$	154	223 ± 11
$CuCl_2 \cdot 2\ H_2O$	142 ± 18	
CuCl[d]		231 ± 22
$MgCl_2 \cdot 6\ H_2O$	113	
$CoCl_2 \cdot 6\ H_2O$	129	
$NiCl_2 \cdot 6\ H_2O$	108	
$MnCl_2 \cdot 4\ H_2O$	60	

[a] Results of duplicated experiments conducted once or twice at different time intervals.
[b] 100% = 4.025 μg. ± 0.42 per reaction mixture.
[c] 100% = 1.805 μg. ± 0.27 per reaction mixture.
[d] Did not totally dissolve in water at 0.5m*M*.

of Fe^{2+} or Cu^{2+} to a boiled enzyme preparation did not result in an epoxidation of aldrin.

In additional tests, NAD, NADP, and NADPH were added to reaction mixtures at 1×10^{-4} *M*. Recoveries of dieldrin were similar to controls, indicating that these cofactors had no effect on the rate of epoxidation of aldrin. This was not surprising, since NAD, NADP, and NADPH are usually cofactors of microsomal or mitochondrial enzymes, while in pea roots over 50% of the enzyme activity is located in the soluble fraction.

Enzyme Inhibitors. Manganese dichloride, added in a single experiment at 1m*M* to a reaction mixture, inhibited the epoxidation of aldrin (Table II). Further tests were conducted with two different enzyme preparations in which either $MnCl_2$ at 1m*M*, sesamex at 5×10^{-4}*M*, or SKF 525-A at 5×10^{-4}*M* was added to the reaction mixture. The enzyme sources were the soluble fraction obtained at 105,000 G from a pea root homogenate, or the protein fraction prepared at an 0 to 70% ammonium sulfate saturation from another soluble fraction. The amounts of dieldrin recovered from these incubation mixtures were 70 ± 10% ($MnCl_2$), 26 ± 1% (sesamex) and 20 ± 5% (SKF 525-A) of those amounts that were recovered from control reaction mixtures. This indicated an inhibition of 30, 74, and 80%, respectively. Sesamex inhibited the epoxidation of aldrin with pea root enzymes. This phenomenon has also been reported with houseflies (Sun and Johnson, 1960) and with soils and soil microorganisms (Lichtenstein *et al.*, 1963). SKF 525-A, which is known as an inhibitor of microsomal enzymes, also inhibited the aldrin epoxidizing enzyme obtained from pea roots.

Heptachlor and Isodrin as Substrates for Pea Root Oxidase. HEPTACHLOR. Three experiments were conducted in duplicate in which the substrate aldrin was replaced by heptachlor. In one test, the enzyme source was the protein fraction obtained at a 0 to 70% ammonium sulfate saturation of a root homogenate; in the second test it was the soluble fraction obtained after centrifugation of a root homogenate at 105,000 G; and in the third test it was the pellet as well as the supernatant obtained after centrifugation of a root homogenate at 105,000 G. Aldrin was used as a control in these experiments and dieldrin was produced in each of these control tests. However, no heptachlor epoxide could be detected in any of the reaction mixtures in which heptachlor had been used as the substrate under identical experimental conditions.

ISODRIN. To test further the specificity of the pea root enzyme, isodrin—the endo-endo isomer of aldrin—was used as the substrate. Duplicated amounts of 0.5, 1.0, or 2.0 ml. of a pea root homogenate were used as the enzyme source and diluted each to 10 ml. with buffer solution. Four hundred micrograms of isodrin was added in 20 μl. of ethyl alcohol to these mixtures. As controls, aldrin was added to two additional reaction mixtures which contained 0.5 or 1.0 ml. of the homogenate. Although 6.0 and 10.0 μg. of dieldrin were produced in these controls, no endrin—the endo-endo isomer of dieldrin—was produced from isodrin, as determined by gas-liquid chromatography. To verify these findings by another method, the total extract from each of two incubated reaction mixtures containing 2 ml. of homogenate and isodrin was concentrated and tested by thin-layer chromatography on aluminum oxide G-coated glass plates. After the chromatograms had been developed with a mixture of isooctane and diethyl ether (7 to 3) they were sprayed with reagents as described by Mitchell (1957), followed by exposure to UV light for 10 minutes. The minimum amount of endrin (R_f 0.62) which could be detected by this method was 0.5 μg. Although none of the reaction mixtures evaluated by chromatography contained endrin (R_f 0.62), a compound of unknown character was visualized (R_f 0.34) in addition to isodrin (R_f 0.71).

CONCLUSION

Three major findings relative to the differences between enzymes from animals and pea plants resulted from this study. The aldrin epoxidizing enzyme(s) obtained from pea roots is different from those systems described in animal tissues by Nakatsugawa *et al.* (1965), and by Lewis *et al.* (1967). These authors reported that the epoxidizing enzyme is of microsomal origin, while in pea roots most of the activity was found in the soluble fraction. Heptachlor epoxide was produced from heptachlor with liver homogenates (rat and rabbit) and with homogenates of houseflies, and endrin was produced from isodrin with rat liver microsomes. The pea root enzyme or enzymes, though, appear to be specific for aldrin and did neither epoxidize the endo-endo isomer of aldrin nor heptachlor. Finally, cofactors such as NAD, NADP, and NADPH did not effect the epoxidation of aldrin with subcellular fractions of pea roots.

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