Formation of Polar Metabolites from Aldrin by Pea and Bean Root Preparations

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The gas chromatographic analysis of the products from combined replicate incubations of aldrin with pea or bean root preparations has indicated the presence of a series of related polar metabolites (including dieldrin). All of the polar metabolites (from beans) detected by gc analysis were confirmed by chemical ionization-mass spectrometry. *In vitro* metabolism studies with synthetic standard polar metabolites showed that none of the systems appear to be intermediate in the formation of others. It is proposed that all but one (aldrin alcohol) of the polar metabolites could arise *via* a common intermediate cation or equivalent radical resulting from attack on aldrin by an activated electrophilic oxygen species. The enzyme behavior is similar to the mixed function oxidase type, with an apparent stimulation by NADPH and formation of a product which could be the result of a NIH shift. The formation of the aldrin alcohol suggests that aldrin can be attacked by at least one other enzyme which can incorporate the elements of water.

Aldrin is among the more widely used chlorinated polycyclodiene pesticides that can contaminate our environment. While its fate in a variety of organisms is fairly well understood (Brooks, 1973), its fate in plants has not been fully elaborated. The chlorinated polycyclodiene pesticides and their conversion products found in plants should be scrutinized as environmental agents since such compounds found in edible plants may be directly ingested by the human population. Some of these compounds may be further altered before human consumption by food processing procedures. Thus, elaboration of metabolic pathways of pesticides in plants may be of considerable importance. There are several advantages for employing *in vitro* methods in the study of pesticide metabolism, some of which have been described by Yu *et al.* (1971).

It is clear that the refinement of *in vitro* techniques in plants for studying the degradation of pesticides would be a valuable asset in dealing with the environmental hazards of pesticide residues. The purpose of this work was to assess the nature of the polar metabolites of aldrin in plant root systems which have been shown (Lichtenstein and Corbett, 1969; Mehendale *et al.*, 1972; Yu *et al.*, 1971) to contain sufficient enzyme activity of the oxidative type for practical use in pesticide metabolism studies. In addition, analytical methodology to permit confident structure assignments of the low level degradation products was sought. No attempt had been made to quantify completely the formation of the metabolites, but it is felt that after refining *in vitro* techniques with the aim of producing the highest possible levels of degradation products, the present analytical methodology will be more than adequate for quantitation purposes. In addition, such studies should reveal any similarities or differences in the metabolic patterns for aldrin in plant and animal systems, as well as afford information of general mechanistic interest.

MATERIALS AND METHODS

Standards. Aldrin and dieldrin used were analytical grade samples obtained from Applied Science Labs, Inc. Dihydroaldrin (DHA), 4-oxo-4,5-dihydroaldrin (aldrin ketone-AK), *exo*-4-hydroxy-4,5-dihydroaldrin (*x*AA), *endo*-4-hydroxy-4,5-dihydroaldrin (*n*AA), *trans*-4,5-dihydroxy-4,5-dihydroaldrin (TAD), and *cis,exo*-4,5-dihydroxy-4,5-dihydroaldrin (CAD) were prepared according to published procedures (McKinney *et al.*, 1971) and have well defined stereochemical structures.

Plant Materials, Root Preparations, and Incubation Procedure. Beans (*Phaseolus vulgaris*, L., Dwarf Horticulture variety) and peas (*Pisum sativum*, L., Alaska Wilt Resistant variety) were raised and active root preparations were made as reported earlier (Mehendale *et al.*, 1972). Incubation procedures (2 g of root material was used for each crude homogenate preparation) were as previously described (Mehendale *et al.*, 1972) except for the use of ethanol or in some cases dimethyl sulfoxide to solubilize the compound for introduction into the incubation mixture. The procedures utilized Polyclar AT as well as a *p*-aminobenzoic acid (Mehendale, 1973) to enhance enzyme activity. The amount of aldrin incubated varied from 20 to 400 μg to as much as 3 mg, depending upon the experimental design. Boiled enzyme preparations incubated with substrate were used as controls. All incubations were done in duplicate or greater.

Extraction and Analysis. Reaction quenching and extraction were also conducted as previously described (Mehendale *et al.*, 1972). In the case of the 20-μg incubations, hexane–acetone (9:1) was used as extraction solvent to facilitate extraction of highly polar materials which may be tightly enzyme bound (Lichtenstein and Corbett, 1969). The pooled extracts were adjusted to an appropriate final volume (1–10 ml) by evaporation under a nitrogen stream and dried over anhydrous sodium sulfate. Either a portion or all of this dried solution was evaporated to dryness and the residue was silylated according to the published procedure (McKinney *et al.*, 1972) utilizing europium nitrate to selectively remove pyridine and other impurities prior to gas chromatography (gc). The benzene solution of silylate products, including unaltered aldrin, dieldrin, and ketonic products, was analyzed on a Varian Aerograph Model 1868-40 chromatograph equipped with a tritium foil electron capture detector and connected to a 1-mV recorder. In some cases it was desirable to selectively remove excess aldrin by adding 0.1 to 0.3 ml of an ether solution of osmium tetroxide to the benzene solution of silylated products, followed by gentle evaporation and redissolution in hexane, leaving behind the insoluble aldrin osmate ester. (This procedure can not be used for diol analysis since some *cis*-aldrin diol (CAD) is formed from hydrolysis of the osmate ester.)

The gas chromatographic columns used were standard stainless steel columns, ⅛ in. × 5 ft, packed with 5% QF-1 on 100/120 mesh Varaport 30, 3% SE-30 on Varaport 30 (100/120 mesh), or 10% OV-225 on 80/100 mesh Chromosorb W, HP. Gas chromatographic conditions were: oven temperature at 185°; inlet temperature at 205°; detector temperature at 225°; and nitrogen carrier gas flow rate at 35–45 ml/min for QF-1 and SE-30 and 70 ml/min for OV-225. High purity or pesticide quality solvents were used throughout.

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Table I. Thin-Layer Chromatography of Aldrin, Dieldrin, Dihydroaldrin, and Polar Metabolite Possibilities

Mobile phase	Standards		R_f					
	Aldrin	Dieldrin	DHA	TAD	CAD	xAA	nAA	AK
Methylene chloride	0.82	0.78	0.77	<0.05	0.09	0.47	0.38	0.49
Ether–hexane (1:1)	0.90	0.77	0.91	0.35	0.47	0.60	0.60	0.44
Hexane–acetone (1:1)	0.79	0.73	0.79	0.51	0.65	0.67	0.67	0.69
Ether–hexane (9:1)	0.79	0.71	0.79	0.35	0.54	0.63	0.66	
Benzene–ethyl acetate (3:1)	0.79	0.71	0.71	0.21	0.44	0.60	0.61	0.61
Hexane–acetic acid (3:1)	0.70	0.50	0.70	0.03	0.09	0.20	0.20	
Benzene–acetic acid (1:1)	0.94	0.91	0.91	0.79	0.79	0.82	0.90	

Table II. Gas Chromatographic Analysis of Standards and Polar Metabolites of Aldrin [Retention Time (min)]

Standards (as silyl ethers except AK and dieldrin)		Polar metabolites from prep tlc	
		Roots of dwarf beans	Roots of Alaska peas
	5% QF-1		
xAA	2.19	2.19	2.19
TAD	2.61	2.61	2.61
Dieldrin	3.06	3.00	3.00
CAD	3.36	3.36	3.42
Unknown		3.78	3.78
AK	4.32	4.38	4.44
	3% SE-30		
AK	6.84	6.90	6.84
Dieldrin	7.08		7.20
Unknown		7.92	7.80
xAA	9.45	9.33	9.33
TAD	14.0	13.9	13.9
CAD	17.4	17.1	
	10% OV-225		
Unknown		14.0	
TAD	15.6	15.7	15.6
xAA	17.0	17.3	17.3
CAD	24.6	24.6	24.6
Dieldrin	31.9	32.3	31.9
AK	41.3	41.6	41.3

Table III. mμMoles of Dieldrin and *trans*-Aldrindiol Produced by Alaska Pea Root Crude Homogenate[a] with Varying Concentrations (*M*) of Added *p*-Aminobenzoic Acid (PABA)

PABA	No NADPH		NADPH	
	Dieldrin	*trans*-Diol	Dieldrin	*trans*-Diol
0	45.4	6.27	46.8	7.39
10^{-2}	33.9	3.56	36.0	3.63
10^{-3}	47.6	6.02	51.7	6.77
10^{-4}	55.5	7.02	57.2	8.77
10^{-5}	46.1	7.52	45.4	8.02
10^{-6}	45.6	6.8	45.8	7.26

[a] Incubated with 400 μg of aldrin in 20 μl of EtOH in sodium phosphate buffer, pH 6.5, for 4 hr at 37° in a metabolic shaker. NADPH, where added, 2 μmol/incubation. Total volume, 4 ml. Desired concentration of PABA was added to the flask. Crude homogenate was prepared using Polyclar AT at 0.25 g/g of roots (Mehendale *et al.*, 1972).

The samples used for mass spectral identification of diols were obtained by combining the products of 28 replicate incubations of 400 μg of aldrin followed by preparative thin-layer chromatography (tlc) on precoated (250 and 500 μ) silica gel G plates developed with methylene chloride to remove excessive amounts of aldrin and dieldrin. However, later experiments showed that 12 replicate incubations of 400 μg of aldrin followed by microcolumn chromatography on Silicar CC-7 sufficed to enable mass spectral confirmation of all of the metabolites indicated by ecgc except the *cis*-aldrin diol (CAD). The column chromatography of these incubation extracts includes hexane elution to remove unmetabolized aldrin, followed by hexane–acetone (9:1) to remove mostly dieldrin and lastly hexane–acetone (1:1) to remove the 'ols and 'ones. The combined extracts for a given fraction were evaporated to a residue which was silylated as previously described. One to 3 μl of the benzene solution containing the silylated products was used for analysis by combined gas–liquid chromatography and chemical ionization mass spectrometry (gc–ms).

Both a 10% OV-225 column and a 1:1 mixed column of 10% OV-225 and 3% QF-1 on 80/100 Gas Chrom Q were employed for gc–ms analysis. The chemical ionization system consisted of a Varian Aerograph Model 1400 gas chromatograph interfaced to a Finnigan 1015C Quadrupole chemical ionization mass spectrometer, with the reagent methane gas flow adjusted to hold 600 μ of pressure. Best results were obtained when the ionizer temperature was maintained between 50–75° by manifold heat transfer. Immediately following injection of the sample onto the column at 160° with the ionizer off, the column temperature program from 160–240° at a rate of 10°/min was initiated. After 5.5 min the ionizer was turned on simultaneously with initiation of the computer control for collection of data (System 150 data collection system).

RESULTS

The gc analysis of the polar hydroxylic metabolites of aldrin was most reproducibly accomplished by silylation prior to injection according to previously reported methods (McKinney *et al.*, 1972). The silylation procedure used has been shown not to alter the gc properties of such compounds as dieldrin and the aldrin ketone. This procedure was particularly desirable since both the *cis*-aldrin diol and *endo*-aldrin alcohol can undergo epimerization and decomposition to form the configurationally more stable *trans*-aldrin diol and *exo*-aldrin alcohol, respectively, among other products when direct gc analysis is attempted. Other experiments showed that a portion of the silylation solution could be treated with a standard osmium tetroxide solution to quantitatively remove excess aldrin from the reaction mixture before gc analysis. This was advantageous in searching for dihydroaldrin as a possible reductive metabolite of aldrin. On the other hand, the 10% OV-225 column separated DHA (11.6 min) from aldrin (9.1 min) by well over 2 min, enabling the detection of DHA in the presence of equivalent amounts of aldrin.

In order to obtain sufficient amounts of polar metabolites other than dieldrin for mass spectral analysis, it was necessary to combine the products of at least 12 to 28 replicate incubations of 400 μg of aldrin. Excessive amounts of aldrin and dieldrin were removed by preparative tlc or microcolumn chromatography. There is an undeterminable loss of the chlorinated polar metabolites that strongly adhere to the silicic acid material during chromatography; however, this can be minimized by extracting or eluting with polar solvents that can inactivate the binding sites such as hexane–acetone and hexane–acetic acid mixtures. As seen from Table I, all of the polar metabolite possibili-

ties are best separated from aldrin, dieldrin, and DHA by the use of methylene chloride as the mobile phase. No attempt was made to identify the polar metabolites by cochromatography using the authentic standards and various combinations of the mobile phases listed since the detection limits for these materials on tlc are much higher than gc and adequate quantities of metabolites were not available. Nevertheless, these R_f data should prove beneficial at a later date for this purpose, as well as for preparative tlc.

A portion of the silylated product solution from the combined incubations was used to analyze for polar metabolites on three different gas chromatographic columns possessing varying and selective separation properties for the standard metabolite possibilities. The retention times obtained for standards as compared with those derived from the products of both Dwarf bean and Alaska pea incubations are shown in Table II. Although other peaks were found in the chromatograms of the metabolites, those given are the major components of which, in most cases, the diols constituted the major portion.

A study of the retention time data in Table II clearly indicates that the retention times observed for the metabolites are in good agreement with those of standard dieldrin (some dieldrin remains even after attempted removal by preparative tlc), aldrin ketone, *cis*- and *trans*-aldrin diols, and *exo*-aldrin alcohol. The *endo*-aldrin alcohol was not found as a major component in the metabolism samples, but due to its similar chromatographic behavior to the *exo*-aldrin alcohol, it is possible that the peak attributed to *x*AA may contain some small amount of *n*AA.

It is also interesting that an unknown major component is found on all three columns. The relative retention time of this unknown (dieldrin = 1.00) on the 5% QF-1 column calculates to be 1.3, which is in fair agreement with the 1.4 value reported (McKinney *et al.*, 1972) for the silyl ether of the major fecal metabolite (C-12 hydroxydieldrin) of dieldrin in the rat on the same column. Unfortunately, a sufficient quantity of this metabolite was not available for use as a comparative standard nor was its presence confirmed by gc–ms.

Aldrin ketone and dieldrin are difficult to distinguish on the 3% SE-30 column; however, they are clearly distinguishable on the other two columns. Although 10% OV-225 affords broad peaks and requires greater amounts of sample, it separates most of these compounds by 7 min or longer. Therefore, from the standpoint of gc–ms analysis, 10% OV-225 would permit return to base line before the next component begins to elute, as well as allow plenty of time (10 min or more) for procedural impurities to elute from the column before initiating the computer-controlled analysis of the desired components. However, 10% OV-225 mixed with 3% QF-1, while giving the same elution pattern as 10% OV-225, gave overall shorter gc runs with sharper peaks. The high liquid phase load did not lead to excessive column bleed if proper conditioning was done (column is conditioned until a stable saturation point is achieved). Since the gc runs were reasonably short, no attempt was made to study the retention properties of lower liquid phase loads.

Although in some experiments Polyclar AT alone was used to enhance enzyme activity, in these studies both Polyclar AT and *p*-aminobenzoic acid (PABA) were used. PABA has been previously (Mehendale, 1973) shown to reproducibly enhance dieldrin production; however, a statistical treatment of the data was not done. Table III shows the reproducible effects of PABA at varying concentrations on the production of *trans*-aldrin diol (TAD) as well as dieldrin, both in the presence and absence of NADPH in Alaska pea root crude homogenates. Other peaks were discernible in the ecgc analyses of these incubation extracts; however, the peak measured was the major peak attributable to nonderivatized *trans*-aldrin diol.

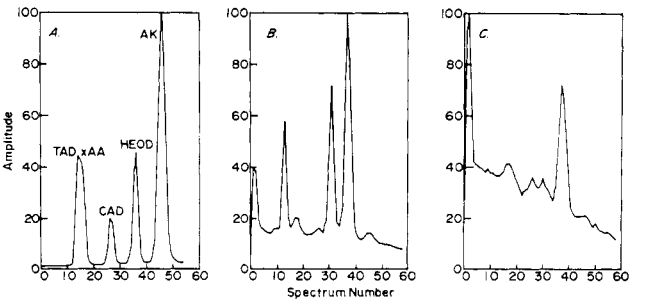

Figure 1. Reconstructed gas chromatogram on 10% OV-225 + 3% QF-1 of: (A) standard TAD, *x*AA, and CAD silyl ethers mixed with dieldrin (HEOD) and oxodihydroaldrin (aldrin ketone, AK). (B) Silylated products from aldrin bean root incubations. (C) Silylated products from aldrin bean root incubations with limited mass search 69–75.

Gc–Ms Analysis. The remaining total solutions of the silylated products from the combined pea and bean root incubation were subjected to gc–ms analysis. Repeatedly, however, the pea root extracts failed to show detectable quantities of the polar metabolites other than dieldrin and TAD. The bean root extracts, on the other hand, did contain detectable quantities of the polar metabolites evidenced by ecgc analysis. Some problems were encountered with CAD when the replicate incubations were smaller in number, as previously described. The computer reconstructed gas chromatogram (rgc) of the silylated combined extracts (see Figure 1b) from the aldrin bean incubations was compared with the rgc of the standard silyl ether derivatives (see Figure 1a) of the alcohols, along with dieldrin and the aldrin ketone. This comparison indicated that masking quantities of impurities were still present. Further purification of the samples at this point was not attempted since further loss of metabolites would result, in addition to possible contamination by materials contained in silica gel. The level of impurities was sufficiently low that they did not interfere with computer-limited mass searches. It can be seen from Figure 1a that the silyl ethers of TAD and *x*AA appear to elute together on this column. Limited mass searches show that the TAD silyl ether elutes slightly ahead of *x*AA.

A study of the mass spectra of the standard silyl ethers under the conditions used revealed that all were characterized by a base peak or second most abundant ion of *m/e* 69. On other occasions, the base peak was observed to be 79, as is found in the normal electron impact mass spectra for these compounds (*m/e* 73 for silyl ethers), but the 69 base peak appears to be characteristic of a variety of chlorinated hydrocarbons under certain chemical ionization conditions obtained with our instruments. The computer reconstructed gas chromatogram with a limited mass search (*m/e* 69–75) is shown in Figure 1c. This chromatogram is clearly different from the total ion chromatogram shown in Figure 1b and indicates the presence of mostly dieldrin, along with other minor components. There was at least one component (spectrum numbers 28–30) that contained some ions in the 69–75 range which was obviously not derived from aldrin.

Chemical ionization mass spectra for most compounds are characterized by increased intensities of fragments at or near the parent ion. The mass spectra of the standard mixture of the suspected polar metabolites indicated that all except aldrin ketone are characterized by the M − Cl fragment being the first or second most abundant fragment in the spectra. For aldrin ketone the parent ion itself was more abundant than the M − Cl fragment. Limited mass searches for these fragments (diols, M − Cl, 505–511; alcohol, M − Cl, 417–423; dieldrin, M − Cl, 343–349; aldrin ketone, M, 378–384) confirmed that all of the expected compounds were present except for the *cis*-aldrin diol. However, earlier mass spectral analysis with a

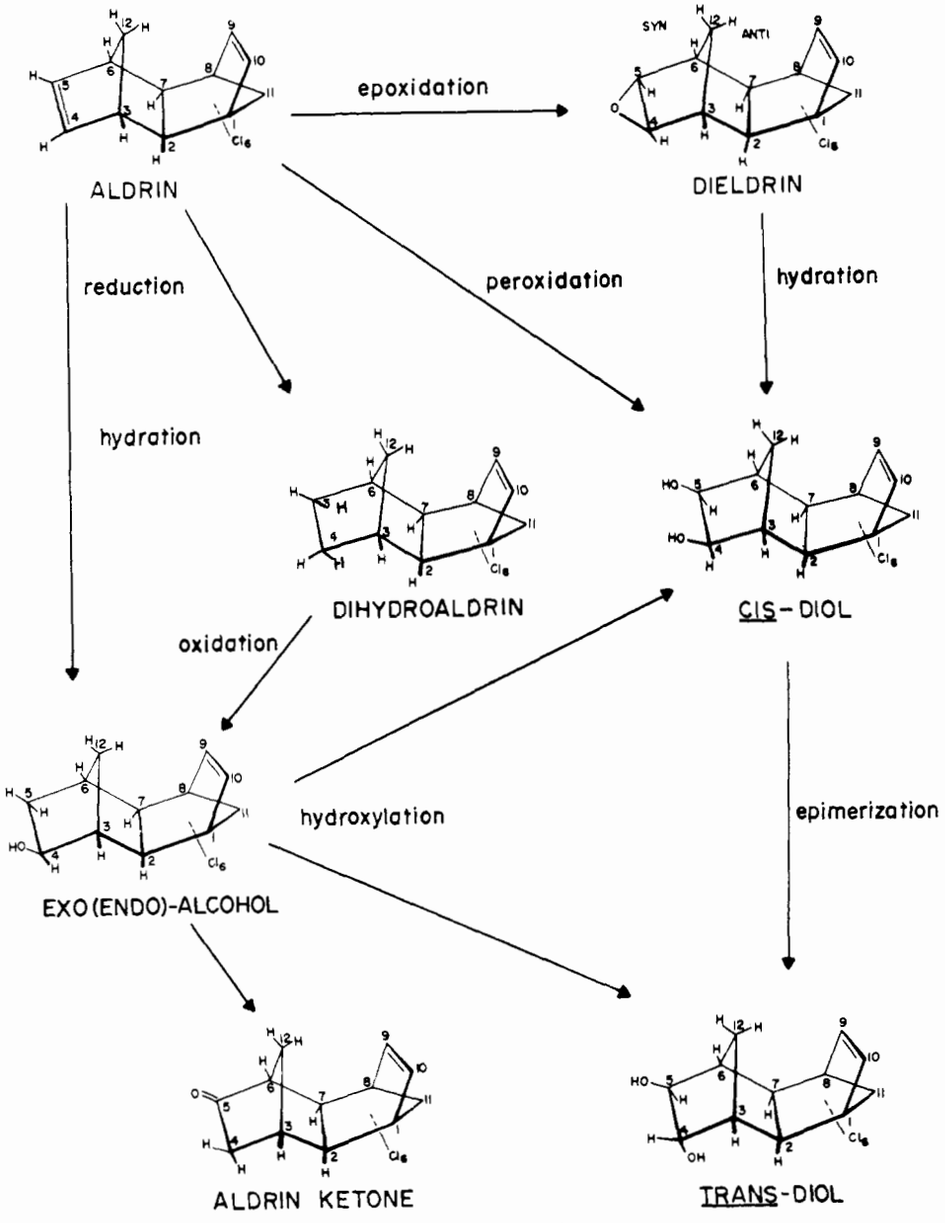

Figure 2. Possible metabolic pathways of aldrin in pea and bean root preparations.

limited mass search of the combined extracts from 28 replicate incubations confirmed that CAD is a product of the metabolism of aldrin. Failure to detect the *cis*-aldrin diol when only 12 replicate incubations or less is used is not surprising since survival time of its disilyl ether in complex mixtures is reduced by the steric compression of the two cis-oriented bulky trimethylsilyl groups. In solutions of the standard silyl ether derivatives of the alcohols and diols, the CAD silyl ether is clearly the most unstable with time. Further elaboration of both the electron impact and chemical ionization mass spectra of these systems will be reported elsewhere (McKinney and Oswald, 1973).

Study of Possible Intermediary Metabolites. Dieldrin was the major metabolite found in both bean and pea root incubations, occurring in some 7–10 times greater concentrations than the combined total 'ols' and 'ones' concentration as estimated by gas chromatography. The aldrin diols were present in somewhat larger relative concentrations than the remaining polar metabolites. Therefore, it was conceivable that those metabolites present in lower relative concentrations might be intermediate in the formation of those present in larger amounts. Figure 2 illustrates the several possible intermediary pathways which could account for the products isolated. Dieldrin intermediacy in the formation of the *trans*-aldrin diol has been previously (Yu *et al.*, 1971) tested and is not pictured here; however, it was again tested as a possible intermediate in CAD as well as TAD formation. Two levels of alternate substrate were incubated with root preparations known to be active in metabolizing aldrin; the normal 400-μg level as well as a 20-μg level (a range approaching that found in the normal incubations with aldrin as substrates) was used. Incubation conditions were identical to those given in Table III except without PABA. No metabolism was detected for any of the polar compounds isolated from aldrin metabolism with the possible exception of the *exo*-aldrin alcohol, which afforded trace amounts of a compound with a retention time identical to aldrin ketone.

DISCUSSION

Gas chromatographic analyses of the products from aldrin incubations with bean or pea root extracts have indicated the presence of a series of related polar metabolites other than dieldrin, of which TAD and CAD appear to be predominant. The presence of all the polar metabolites

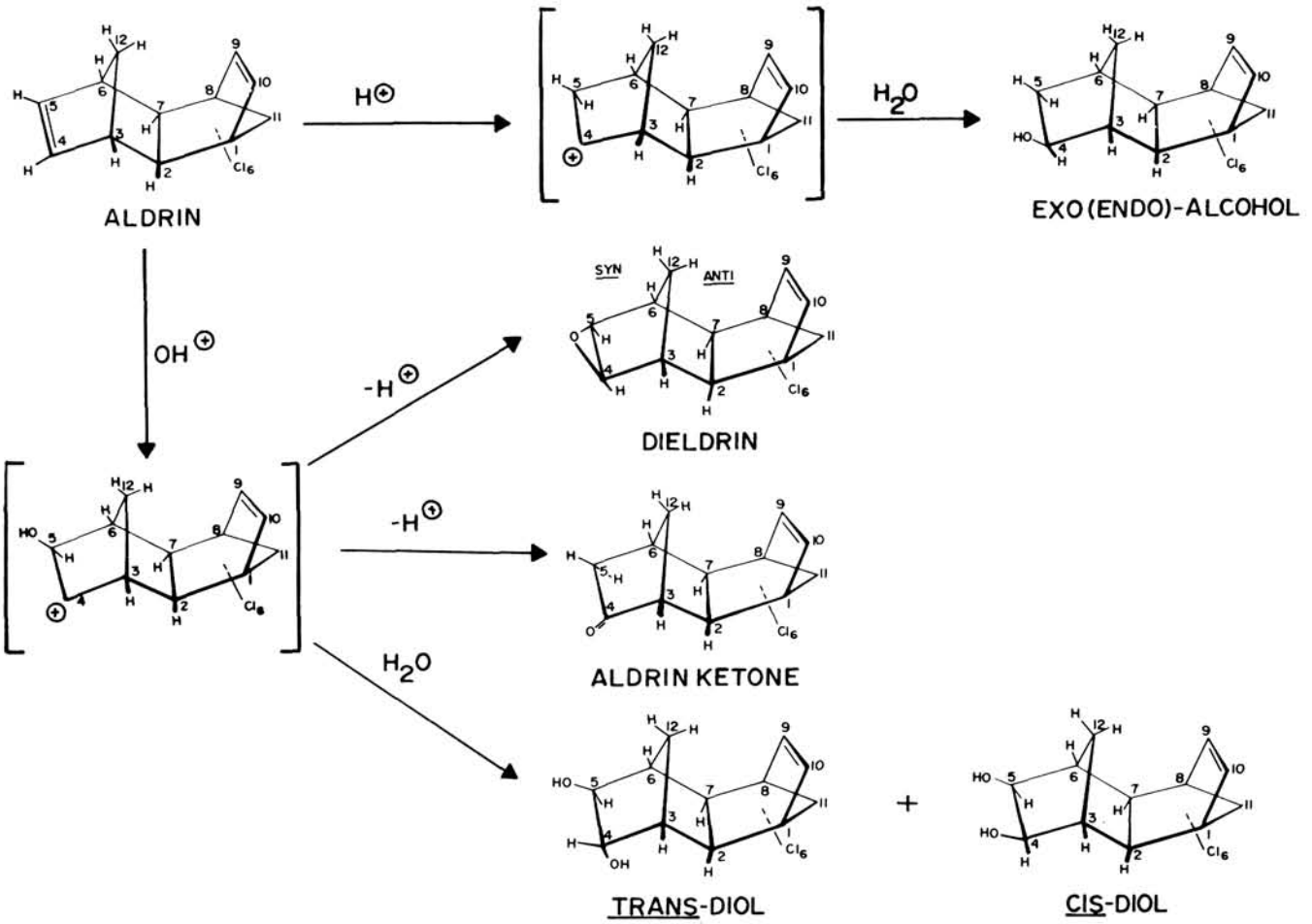

Figure 3. Common ion intermediacy in the formation of polar metabolites of aldrin in pea and bean root preparations.

detected was confirmed by chemical ionization mass spectrometry (products from bean root incubations of aldrin were utilized for ms confirmation since relatively larger amounts could be isolated). It should be noted that the direct gc determination of TAD as a metabolite can be misleading since nonderivatized CAD undergoes appreciable conversion under some gc analysis conditions to material chromatographically identical to TAD. Thus, somewhat higher levels of TAD would result with the apparent absence of any CAD. Root preparations spiked with CAD followed by silylation of the extracts indicated that silylation is complete since no TAD was detected under gc conditions which appear to epimerize nonderivatized CAD. Nevertheless, mass spectral analysis of the silylated incubation products has confirmed the presence of both the aldrin diols in nearly equal concentrations, albeit in low yields based on starting substrate, along with aldrin alcohol and aldrin ketone. In addition, when one 3-mg incubation of aldrin was performed and analyzed by mass spectrometry after the pretreatment with osmium tetroxide, an isomer of dieldrin was found whose spectral properties more closely resembled those of dieldrin than they did the aldrin ketone. It is conceivable that the *endo*-epoxide isomer of dieldrin could have resulted from endo attack on the aldrin double bond.

In vitro incubation studies with synthetic standard metabolites did not provide any information to support the intermediacy of certain ones of these systems in the formation of others. On the contrary, and as suggested previously (Yu *et al.*, 1971) for TAD formation, these polar metabolites may be the result of independent attack on aldrin by differing systems, although their data suggested that the conditions for optimum activity (for dieldrin and TAD formation) were identical. The possibility of CAD formation from dieldrin and subsequent epimerization of CAD to TAD was particularly attractive since recent work (Matthews and McKinney, 1972) has provided evidence that such pathways may be operating in the metabolism of dieldrin by rat liver microsomal systems. On the other hand, the direct formation of CAD *via* peroxidation is equally attractive. Other plausible pathways which can account for aldrin alcohol formation as well as diol formation involve the nonstereoselective hydroxylation of DHA (previously observed in pig liver by Brooks and Harrison, 1969) and aldrin alcohol (endo or exo), respectively, as shown in Figure 2. The failure to detect any metabolism of DHA or otherwise detect its presence in our studies in addition to the unsuccessful attempts to metabolize the *exo*-alcohol, the *cis*-diol, and dieldrin cast considerable doubt on the possibility that such pathways as these are operating. From these results, one infers that the polar metabolites are all formed directly from aldrin *via* a common intermediate derived from one enzyme reaction, or they are all formed from aldrin *via* separate enzyme reactions.

An alternative explanation for the formation of essentially all of the metabolites found, and one in which only one enzyme system may be required, involves the formation of a common cation or equivalent intermediate derived from electrophilic attack of OH^+ or an equivalent species on the double bond of aldrin. This would be analogous to the oxygenation of aromatic compounds to form a common ion leading to several metabolites, including the product of a "NIH shift" (known to occur in plant systems; Russell *et al.*, 1968). The nature of the products formed depends largely on the kinetics and thermodynamic properties encompassing the neutralization and rearrangement of such intermediate ions. Epoxides, alcohols, diols, and ketones, among others, are usually the resulting products. Figure 3 illustrates the intermediacy of a com-

mon ion (the direct transfer of OH^+, although unlikely (Jerina *et al.*, 1971), with generation of a cationoid intermediate serves as an acceptable and reasonable mechanism for this type of enzyme reaction) in the formation of the polar metabolites of aldrin. The aldrin ketone would be the resultant product of a NIH shift. The observed parallel enhancement in the production of dieldrin and *trans*-aldrin diol (TAD) by *p*-aminobenzoic acid, as indicated in Table III, is not inconsistent with the one enzyme proposal. The apparent stimulation by NADPH, which has been previously observed (Yu *et al.*, 1971), suggests a further resemblance to mixed function oxidase systems.

Aldrin alcohol formation is best explained by a simple hydration mechanism involving protonation and neutralization by a water molecule. This is not an uncommon reaction, even from a nonenzymatic viewpoint. Since the aldrin alcohol is not formed with the boiled enzyme blank, there appears to be an enzyme which can incorporate a proton (H^+) or equivalent species, also forming a cationoid-type intermediate. Subsequent neutralization of this intermediate would afford the aldrin alcohol (possibly both isomers (endo and exo) would be formed if the neutralization reaction is nonstereoselective). The apparent absence of an epoxide hydrase in these pea and bean root systems precludes the intermediacy of dieldrin in the formation of the diols and, therefore, simplifies the metabolic picture. It is likely that further studies of the metabolizing abilities of plants will reveal many metabolizing reactions exhibited by animal systems.

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Characterization of Residues on Plants Following Foliar Spray Applications of Benomyl

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This paper reports the results of special tests using radiolabeled benomyl [methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate] and methyl 2-benzimidazolecarbamate (MBC). These tests confirm by chemical means that the systemic fungicide benomyl has adequate stability in typical aqueous suspensions used for foliar applications. After application to plant foliage, benomyl constitutes a major component of the total residue for extended periods. Other than MBC, which also has fungicidal properties, no residues of possible conversion products of benomyl, particularly those which can form under alkaline conditions, were found in these tests. Thus, intact benomyl is available on treated plant surfaces for systemic fungus disease control. This finding supplements work reported elsewhere which has shown that more biologically active compound enters and moves within herbaceous plants when benomyl, rather than MBC, is applied to leaf surfaces.

Numerous recent literature accounts point to the instability of the systemic fungicide benomyl [methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate] under certain conditions. Benomyl in solution and in plants has been reported to degrade to a fungitoxic degradation product known as MBC, methyl 2-benzimidazolecarbamate (Clemons and Sisler, 1969, 1970; Fuchs *et al.*, 1970; Kilgore and White, 1970; Maxwell and Brody, 1971; Ogawa *et al.*, 1971; Peterson and Edgington, 1969a,b; Sims *et al.*, 1969). The conversion of benomyl under alkaline conditions to 3-butyl-*s*-triazino[1,2a]benzimidazole-2,4(1*H*,3*H*)dione (STB) and 2-(3-butylureido)benzimidazole (BUB) has also been described (Ogawa *et al.*, 1971; White *et al.*, 1973).

This paper presents chemical evidence which shows that under simulated commercial use conditions for crop protection benomyl possesses excellent stability, both in aqueous suspension for spraying operations and as a residue on treated plants. It is thus available as an intact material for systemic fungus disease control.

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EXPERIMENTAL SECTION

Chemicals. Analytical standards of benomyl and MBC were provided by E. I. du Pont de Nemours & Co., Inc., Biochemicals Dept., Wilmington, Del. [2-^{14}C]Benomyl (2.86 μCi mg^{-1}, 0.830 μCi μmol^{-1}, H. L. Klopping, unpublished data) had a radiochemical purity of >95% as estimated by dissolving a sample in chloroform, quickly