Mammalian Metabolism and Environmental Degradation of the Juvenoid 1-(4′-Ethylphenoxy)-3,7-dimethyl-6,7-epoxy-*trans*-2-octene and Related Compounds

Sarjeet S. Gill, Bruce D. Hammock, and John E. Casida*

Radiolabeled preparations of 1-(4′-ethylphenoxy)-3,7-dimethyl-6,7-epoxy-*trans*-2-octene (ethyl epoxide), its diene precursor (ethyldiene), and its 6,7-diol (ethyldiol) and 2,3-6,7-diepoxide (ethyl diepoxide) derivatives were subjected to degradation in various biological and photochemical systems. Major identified products formed from the ethyl epoxide are: the ethyldiol and α-hydroxyethyldiol on incubation with mouse, rat, or rabbit liver microsome–NADPH systems and algae; compounds resulting from ether cleavage in living mice; the ethyldiol, ethyl diepoxide, and ethylphenol on exposure to sunlight as residual deposits or in aqueous solution. Comparable studies with the ethyldiene, ethyldiol, and ethyl diepoxide led to tentative identification by cochromatography and sometimes by derivatization techniques of over 25 metabolites and photoproducts. The ethyl epoxide is transformed at varying rates in the systems examined by epoxide hydration, 2,3-epoxidation, α and β oxidation of the ethyl group, and ether cleavage.

The safe and effective use of juvenoids (also known as juvenile hormone mimics and insect growth regulators) in pest insect control requires compounds with an appropriate degree of potency, selectivity, and persistence. 1-(4′-Ethylphenoxy)-3,7-dimethyl-6,7-epoxy-*trans*-2-octene (R 20458 of Stauffer Chemical Co.; the ethyl epoxide) has many of the desired properties (Pallos and Menn, 1972). Studies on the degradation chemistry (Hammock *et al.*, 1974a), metabolism in insects (Hammock *et al.*, 1974b) and rats (Gill *et al.*, 1972; Hoffman *et al.*, 1973), and photochemical fate (Gill *et al.*, 1972) of the ethyl epoxide establish that it is transformed largely by reactions occurring at the expoide moiety and the ethyl group although oxidation of the 2,3-double bond and ether cleavage are also important.

In the present study the ethyl epoxide, the nonepoxidized intermediate in its synthesis (the ethyldiene), and its 2,3-epoxide (ethyl diepoxide) and 6,7-diol (ethyldiol) derivatives are used as model compounds to evaluate the mammalian and algal metabolism and photochemical fate of aryl geranyl ether epoxides and related compounds.

MATERIALS AND METHODS

Chemicals. The reference compounds are designated by trivial names shown in Figure 1. The geranyl ethers are referred to as dienes, the 6,7-epoxidized derivatives as epoxides, the 6,7-dihydroxy compounds as diols, and the 2,3-6,7-diepoxidized compounds as diepoxides. Additional modifications of the geranyl moiety are designated as shown in Figure 1. In the cyclic compounds, thf and thp refer to tetrahydrofuran and tetrahydropyran derivatives, respectively. The 4-phenyl substituents include ethyl, α- and β-hydroxyethyl, aceto, carboxymethyl, carboxy, hydroxy, and nitro. The synthesis and the chromatographic and spectroscopic properties of each of these compounds are given by Hammock (1973), Hammock *et al.* (1974a), and Singh (1973). While only those compounds found to be identical with metabolites or photoproducts are shown in Figure 1, many others were used in arriving at these identifications.

The radioactive compounds utilized were labeled with ^{3}H or ^{14}C in the phenyl group or with ^{3}H in the 1 position of the geranyl moiety. They are previously described (Gill *et al.*, 1972; Hammock, 1973; Hammock *et al.*, 1974b; Kalbfeld *et al.*, 1974; Kamimura *et al.*, 1972) with the exception of the [*phenyl*-^{14}C]acetoepoxide, -acetodiol, and -ethyltetrahydrofurandiols, each of 17 mCi/mmol (Singh, 1973). The radiochemical and isomeric purities in each case were >98% based on gas–liquid chromatography (glc) and/or thin-layer chromatography (tlc) and radioautography with the following exceptions: the [*phenyl*-^{14}C]ethyltetrahydrofurandiols contained 10–25% of the α-[^{14}C]hydroxyethyl diepoxide; the [*geranyl*-^{3}H]ethyldiene and [*geranyl*-^{3}H]epoxide consisted of both trans and cis isomers in a 16:9 ratio.

Chromatography and Derivatization Studies. Silica gel F_{254} chromatoplates (20 × 20 cm, 0.25 mm thickness, EM Laboratories, Elmsford, N. Y.) were used for two-dimensional tlc development with solvent systems in the following sequence: benzene–*n*-propyl alcohol, 10:1 or 5:1 mixture (BP 10:1 or BP 5:1) in the first direction; chloroform–methylene chloride–ethyl acetate–*n*-propyl alcohol, 10:10:1:1 mixture (CMAP) in the second direction; carbon tetrachloride–ether, 2:1 mixture (TE) for a second development in the first direction. Procedures for detection of labeled and unlabeled compounds on tlc plates and for quantitation of radiolabeled products by liquid scintillation counting (lsc) are given elsewhere (Hammock, 1973; Hammock *et al.*, 1974a,b; Singh, 1973). Two-dimensional tlc cochromatography was considered to constitute tentative characterization of the labeled metabolites and photoproducts. The identifications were usually substantiated by derivatization studies (Hammock *et al.*, 1974a) involving cochromatography of the derivatives with compounds of known structure. The derivatizing reagents were: *m*-chloroperoxybenzoic acid to form epoxides from dienes and cyclic products from diols; OsO_4–pyridine to convert dienes to diols; 0.05 *N* H_2SO_4 in aqueous tetrahydrofuran to cleave 6,7-epoxides to 6,7-diols and to test for other types of acid-labile compounds; *n*-butylboronic acid (BBA) to form diesters with vicinal diols; lead tetraacetate to oxidize α-hydroxyethylphenyl compounds to acetophenyl derivatives and to cleave vicinal diols to the corresponding aldehydes; Tollen's reagent to oxidize aldehydes to the corresponding acids; diazomethane to methylate carboxylic acids; $NaBH_4$ to reduce aldehydes and ketones and semicarbazide to convert them to semicarbazones; acetic anhydride–pyridine (Ac_2O) to convert primary and secondary but not tertiary alcohols to the corresponding acetates.

Distribution and Metabolism in Living Mice and Rats. Male albino Swiss Webster mice (26–28 g) and Sprague Dawley rats (230–250 g) (Horton Laboratories, Oakland, Calif.) were administered radiolabeled ethyl epoxide in 25 or 50 μl of ethanol, respectively, by the oral route at 1 mg/kg or by the intraperitoneal (ip) route at 1 or 100 mg/kg. Each treated animal was held with food

Division of Entomology and Parasitology, University of California, Berkeley, California 94720.

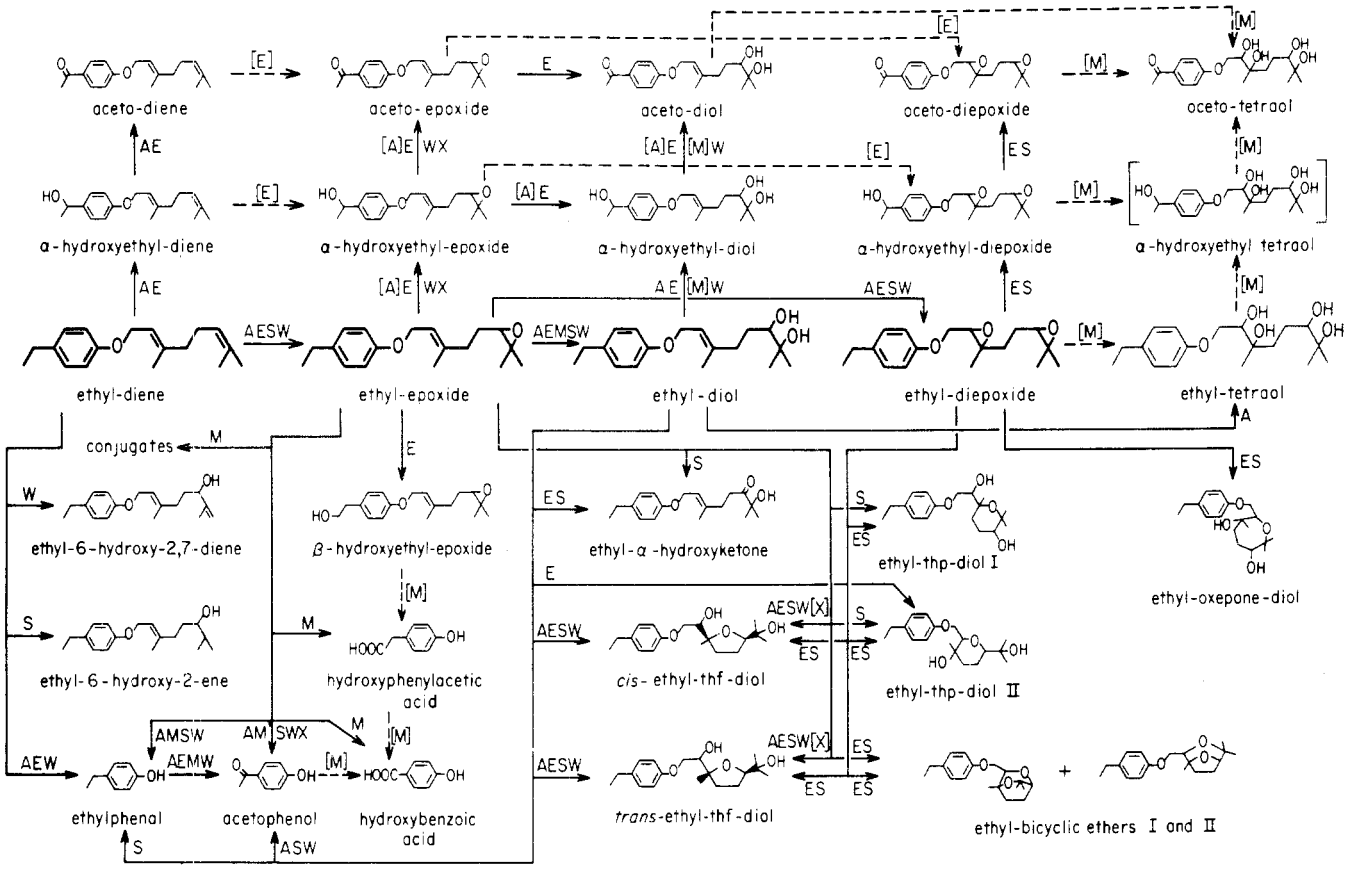

Figure 1. Metabolism and photoalteration pathways for the ethyldiene, ethyl epoxide, ethyldiol and ethyl diepoxide. The metabolites and photoproducts are tentatively identified by cochromatography sometimes with support from derivatization studies as indicated in the text. The compound in brackets is likely to be an intermediate but no authentic sample was available for comparison. Arrows with dotted lines indicate probable pathways based on a series of reactions but they are not confirmed by independent experiments for the individual step. The letters associated with each arrow designate the organism or system shown to carry out the reaction; if the letters are in brackets, the step is not firmly established under the indicated condition. The designations for conditions are as follows: A, algae; E, liver enzymes *in vitro;* M, mice *in vivo;* S, photoalteration on silica gel; W, photoalteration in water; and X, xanthen-9-one-sensitized photodecomposition on silica gel; thf indicates tetrahydrofuran and thp indicates tetrahydropyran.

and water in a metabolism cage for the separate collection of urine, feces, and $^{14}CO_2$ (Krishna and Casida, 1966).

An aliquot of the 0–48-hr mouse urine was extracted with ether (peroxide free) without pH adjustment and after adjustment to pH 1 with 1 *N* HCl to recover the neutral and acidic ether-soluble metabolites, respectively. The rest of the urine was lyophilized to dryness. Then methanol was added to recover the methanol-soluble products which were subjected to direct tlc analysis. Alternatively, the methanol-soluble products, after solvent evaporation, were incubated for 24 hr at 37° with or without glusulase (33,000 Fishman and 3300 Whitehead units) (Calbiochem, La Jolla, Calif.) in sodium acetate buffer (5 ml) (0.1 *M*, pH 4.5). Then each incubation mixture was extracted with ether before and after adjustment to pH 1. Compounds in the ether extract were subjected to cochromatography and lsc.

Mouse feces (0–96 hr) extracts prepared by homogenization in 50% aqueous methanol and centrifugation were evaporated to dryness. Then water was added followed by extraction at pH 7 and 1 with ether. After evaporation of the combined ether extracts, the residual material was partitioned between acetonitrile and hexane; then the products in each phase were analyzed by tlc cochromatography and lsc.

Metabolism *in Vitro* by Rat, Mouse, and Rabbit Enzymes. Tissues from male rats, mice, and New Zealand white rabbits (170, 20, and 1500 g, respectively) were homogenized in 5 vol of sodium phosphate buffer (pH 7.4, 0.1 *M*) using a Teflon-fitted glass homogenizer. The homogenate after filtration through glass wool was centrifuged in sequence as follows: 800*g* for 15 min to precipitate the nuclei and cell debris fraction; 10,000*g* for 10 min to sediment the mitochondrial fraction; 105,000*g* for 60 min to obtain the microsomal fraction; the resulting supernatant was passed through Sephadex G-25 to obtain the "soluble fraction." Each particulate fraction was washed once with fresh buffer prior to use.

The incubation mixtures in phosphate buffer (pH 7.4, 0.1 *M*) (2 ml) contained the following constituents unless otherwise indicated: enzyme (170 mg fresh tissue weight equivalent unless specifically stated otherwise); reduced nicotinamide adenine dinucleotide phosphate (NADPH) or other cofactor (0.5 μmol) or no cofactor; $MgCl_2$ (21 μmol used with liver microsomes only); substrate (0.1 μmol) added last in ethanol (10–30 μl). Following incubation at 37° with shaking in air for 1 hr, the ether-soluble products were analyzed by tlc, radioautography, and lsc. Protein determinations were made by the method of Lowry *et al.* (1951).

Liver microsomes contain enzymes which hydrate the ethyl epoxide to the ethyldiol. For studying their pH dependence and that of the liver soluble enzymes with glutathione (GSH), enzyme preparations (1.0 ml) made in 0.1 *M* KCl were mixed with the appropriate buffer (1.0 ml, 0.2 *M*) and incubated for 1 hr; then the incubation mixture was extracted with ether for tlc analysis of the ethyl epoxide and ethyldiol. Alternatively, the incubated mixture was shaken with 15 ml of toluene containing 0.55% (w/v) of 2,5-diphenyloxazole and then subjected to lsc; if GSH conjugates are formed, they are not expected to extract into toluene so the decrease in organosoluble radioactivity should be due to conjugation reactions. In another experiment, 1.0 ml of human plasma at pH 6.5 or

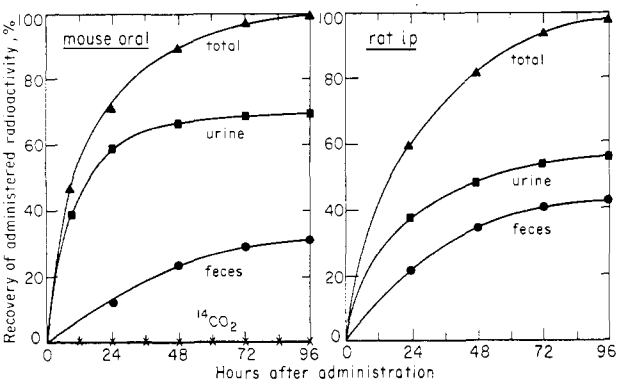

Figure 2. Rate of excretion of radiolabeled products in the urine and feces of mice after oral administration of [*phenyl*-^{14}C]ethyl epoxide and of rats after intraperitoneal administration of [*phenyl*-^{3}H]ethyl epoxide at 1 mg/kg.

rat plasma at pH 7.4 was incubated with the ethyl epoxide and analyzed as above.

Algal Growth and Metabolism. *Effect of Juvenoids on Growth.* Monoaxenic *Chlorella* strain 211 and *Chlamydomonas teinhardii* (Department of Botany, University of California, Berkeley) were cultured in Beijerinck's medium (Stein, 1958) at 18 or 27° under constant fluorescent light (1200 ft-candles at the surface of the culture medium) on a shaking incubator. For assays of growth inhibition, the test compound was added in ethanol (up to 25 μl) to 50 ml of the culture medium in a 125-ml erlenmeyer flask yielding concentrations of 0–100 ppm; then the desired species of alga (*ca.* 1 × 10^8 cells) was added for incubation. Algal growth was monitored by either counting the cells in an aliquot preserved with formalin using a hemacytometer or by monitoring absorbance at 525 nm.

Degradation of Juvenoids by Algae or Chlorophyll in the Presence or Absence of Light. The test compounds were incubated at 2 ppm in living *Chlorella* or *Chlamydomonas* cultures (absorbance 0.30) or in cultures killed by sonication for 1 min. Other controls were uninoculated flasks, inoculated flasks kept in the dark, or flasks containing alfalfa chlorophyll (absorbance 0.82). At various time intervals (12 hr–35 days) aliquots were saturated with $(NH_4)_2SO_4$ and extracted with ether, the acidic and neutral fractions were separated by extraction with 5% aqueous NaOH, and then each fraction was analyzed by tlc developing with petroleum ether–ether (5:1), BP 10:1, or BP 5:1.

Photoalteration. *Photoalteration on Silica Gel.* The ^{3}H- or ^{14}C-labeled compounds were spotted in ethanol (2–10 μl) at the origin of silica gel chromatoplates to yield deposits of 30 nmol/cm^2. The chromatoplates were exposed to sunlight, a sunlamp (275 W, General Electric Co., Cleveland, Ohio), or uv (254 nm, 15 W, Ultra-violet Products, Inc., San Gabriel, Calif.) irradiation or kept in the dark (controls) and then the chromatograms were developed in the dark. In tests with photosensitizers, the candidate compound in chloroform was applied to the tlc plate (15 nmol/cm^2); then the ethyl epoxide was applied (30 nmol/cm^2) to the same area.

To obtain individual photoproducts for spectral analysis, the [*phenyl*-^{3}H]ethyl epoxide (3 g, 400 μg/cm^2) was exposed to sunlight for 10 hr. Then the methanol-soluble products, after solvent evaporation, were dissolved in ether and fractionated by base extraction, tlc, and column chromatography on silicic acid (Singh, 1973).

Photoalteration in Aqueous Media. Solutions of the ethyl epoxide, ethyldiene, or ethyldiol at 0.5 ppm in distilled water were exposed to sunlight through a quartz glass plate or to sunlamp irradiation (with cooling to keep the water at 22–25°) or were kept in the dark. The water was then saturated with NaCl and the ether-soluble products analyzed by tlc and lsc.

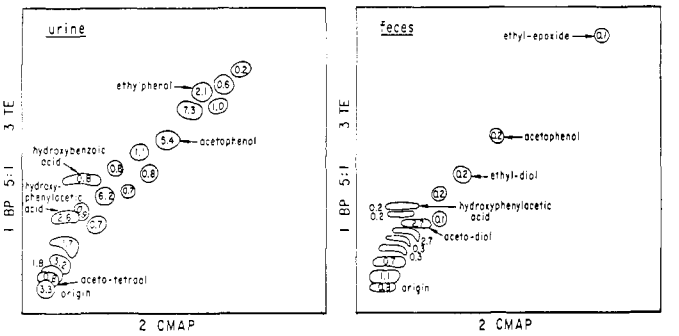

Figure 3. Ether-soluble metabolites excreted in urine and feces of mice after oral administration of [*phenyl*-^{14}C]ethyl epoxide at 1 mg/kg as resolved by tlc. The urine was treated with glusulase. The solvent fronts are the appropriate outlines of the figure.

Biological Activity of Photoalteration Products. The ethyl epoxide at 313 μg/cm^2 was photodecomposed on silica gel by exposure to sunlight for 0–32 hr. After removal of residual ethyl epoxide but not any of the photoproducts from the methanol-soluble materials by use of a silicic acid column (Singh, 1973), each mixture of photoproducts was assayed for morphogenetic activity with *Tenebrio molitor* pupae (Hammock *et al.*, 1974a).

RESULTS

Metabolites of the Ethyl Epoxide in Mice and Rats *in Vivo.* Radioactivity from orally administered [*phenyl*-^{14}C]ethyl epoxide in mice and ip-injected phenyl-^{3}H label in rats, each at 1 mg/kg, appears almost entirely in the urine or feces within 96 hr (Figure 2). Comparable studies utilizing a 100 mg/kg ip dose with mice and rats and a 1 mg/kg oral dose with rats indicated that the urinary radioactivity levels are only 41–75% as high with geranyl-^{3}H as with phenyl-^{3}H label and mice excrete 1.4–2.1-fold more of the ip-administered radioactivity in urine than rats regardless of the labeling position utilized (Singh, 1973). The lower level of urinary radioactivity with the geranyl-^{3}H than with the phenyl-^{3}H label is probably due to extensive cleavage of the ether linkage; half of the tritium is lost on initial hydroxylation at the C-1 position and the other half is removed on exchange or oxidation of the aldehyde released from the unstable C-1 hydroxy intermediate. Phenyl-^{3}H labeled preparations are inappropriate for studies involving oral administration because of exchange due to the acidic conditions in the stomach (Hammock, 1973; Singh, 1973). Radioactivity from oral administration of phenyl-^{14}C label to rats at 1 mg/kg appears rapidly in blood and liver, reaching maximal levels of 11 and 26 ppm of ethyl epoxide equivalents relative to dry tissue weight, respectively, at 0.5 hr and decreasing thereafter at comparable rates for the two tissues (Singh, 1973).

Fractionation of mouse excreta from the 1 mg/kg oral dose of phenyl-^{14}C-labeled compound yields the following ^{14}C distribution in urine and feces, respectively, relative to the administered dose: neutral organosoluble products, 4 and 7%; acidic organosoluble metabolites, 2 and 4%; water-soluble metabolites, 61 and 13%; unextractable residue, 2 and 7%. Incubation of the soluble urinary metabolites (67% of the administered dose) without and with glusulase gives 10 and 44% organosoluble products, respectively, indicating that 34% of the dose is excreted as conjugates, probably ether or ester glucuronides or sulfates. The chemical nature of the uncleaved soluble urinary metabolites (23% of the administered dose) is not known.

The tlc chromatographic patterns, identities, and amounts of the organosoluble urinary (glusulase treated) and fecal metabolites (partitioning into acetonitrile from hexane) from the orally treated mice are shown in Figure 3. Not any of the unidentified metabolites from either

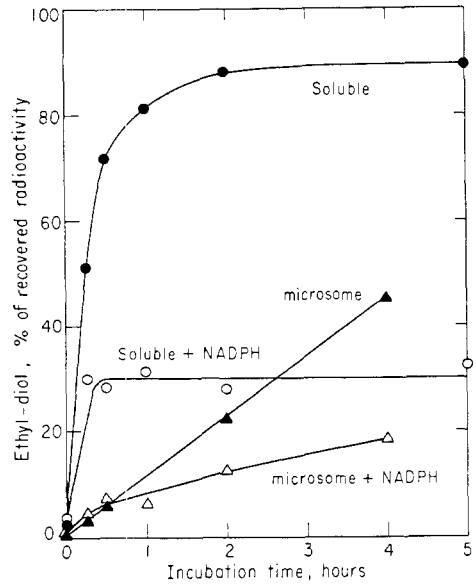

Figure 4. Ethyldiol recovery on incubation of the ethyl epoxide with mouse liver rnicrosome and soluble enzyme systems. The ethyldiol yield is reduced in the presence of NADPH because a portion of the ethyl epoxide and ethyldiol undergo oxidation reactions.

urine or feces cochromatographs with any of the other authentic standards available (Hammock *et al.*, 1974a). The acetotetraol is also identified by cochromatography of its BBA diester. None of the other metabolites have chromatographic properties and BBA reactions appropriate for tetraols, so the carboxytetraol is not present (see Discussion). The identified phenols are base extractable and do not react with OsO_4–pyridine or BBA. Hydroxybenzoic and hydroxyphenylacetic acids are further characterized by tlc cochromatography of their methyl esters. The two major urinary unknowns present in 6-7% amounts are phenols which lack carboxylic acid, vicinal diol, and aliphatic unsaturated groupings based on derivatization studies. The phenol unknown present in 7% amount chromatographs above acetophenol (Figure 3); this chromatographic behavior is anticipated for acetophenol hydroxylated in the 3 position. The phenol unknown present in 6% amount chromatographs as anticipated for α- or β-hydroxyethylphenol but neither of these standards was available for cochromatography.

Metabolism *in Vitro. Enzymatic Hydration of the Ethyl Epoxide.* Rat tissue homogenates convert [*phenyl*-^{14}C]ethyl epoxide to the ethyldiol, the activity being higher for liver and kidney than for brain, lung, spleen, and testes. The relative order of activity of rat liver homogenate fractions at equivalent fresh tissue weight in ethyldiol formation is soluble $\gg$ nuclei and debris $\gg$ microsomes $\geq$ mitochondria. Rat liver microsomes cleave *trans*- and *cis*-[*geranyl*-^{3}H]ethyl epoxides at essentially the same rate to the corresponding diols regardless of the substrate level (0.05–1.0 μmol). Pretreatment of rats with phenobarbital increases liver microsomal activity for hydration of the ethyl epoxide (0.1 μmol) with equal induction for cleavage of the trans and cis isomers. Human and rat plasma are essentially inactive in metabolizing the ethyl epoxide (Singh, 1973).

Enzymatic cleavage of the ethyl epoxide by mouse liver microsomes occurs over a broad pH range (6.0–7.5) with maximum activity at about 6.6. Several oxiranes (cyclohexene oxide, 1,1,1-trichloropropene 2,3-oxide, 1,2-epoxyoctane, 2,2-dimethyloxetane, and styrene oxide) and the ethyl episulfide (the sulfur analog of the ethyl epoxide) at 5 molar equivalents relative to the substrate do not inhibit the rate or extent of hydration of the ethyl epoxide by rat and mouse liver microsomes. However, it is possible

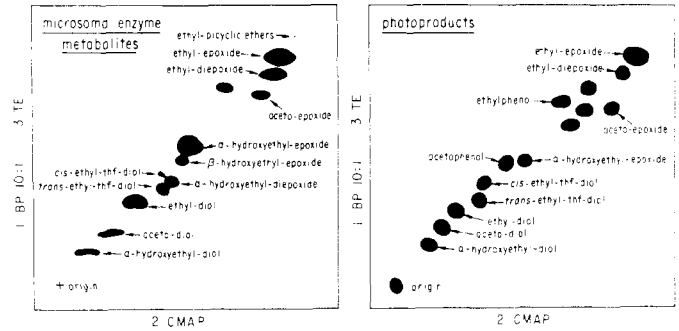

Figure 5. Metabolites of the [*phenyl*-^{3}H]ethyl epoxide formed by the rat liver microsome–NADPH system and photoproducts of the [*phenyl*-^{14}C]ethyl epoxide formed on exposure in water to sunlight for 4 hr as resolved by tlc. The solvent fronts are the appropriate outlines of the figure; thf indicates tetrahydrofuran.

that some of the candidate inhibitors undergo rapid metabolism and do not persist for the 1-hr incubation period involved in the assays.

With mouse liver the soluble fraction is much more active than the microsomal fraction in ethyl epoxide cleavage. The initial rate of ethyldiol formation by either fraction is not greatly altered by NADPH addition but the rate decreases after a few minutes in each case possibly due to competing oxidation reactions when this cofactor is present (Figure 4) as discussed later.

The rat liver soluble fraction is very active in epoxide hydration with minor formation of oxidation products; the activity is lost on heat treatment (Table I). Addition of NADPH to the fresh preparation decreases ethyldiol recovery since other oxidation products are also formed. Nicotinamide adenine dinucleotide phosphate ($NADP^+$) is a less favorable cofactor for formation of these oxidized derivatives while nicotinamide adenine dinucleotides (NADH and NAD^+) are inactive. Cochromatographic evidence for the ethyldiol metabolite is supported as follows: appropriate proton magnetic resonance (pmr) spectrum; ester formation with BBA and Ac_2O; no reaction with $NaBH_4$ or diazomethane; cleavage with lead tetraacetate to 1-[^{14}C](4′-ethylphenoxy)-3-methyl-*trans*-2-hexenal (the ethylaldehyde) which in turn forms a semicarbazone or is oxidized by Tollen's reagent to the corresponding acid. The acetodiol metabolite forms a BBA ester, a monoacetate, and gives the α-hydroxyethyldiol with $NaBH_4$ but fails to react with diazomethane or aqueous acid. The α-hydroxyethyldiol gives a BBA ester and monoacetate but fails to react with diazomethane, $NaBH_4$, or aqueous acid. The tlc pattern of metabolites is the same on comparing the *trans*-[^{14}C]- with the *trans,cis*-[^{3}H]ethyl epoxide except that the *cis*-ethyldiol is detected from the *cis*-ethyl epoxide, further indicating that ether cleavage does not occur under these conditions.

No conclusive evidence was obtained for GSH conjugation of the ethyl epoxide in the presence of the mouse or rat liver soluble fraction. These studies included: the partitioning type product assay and attempts to isolate possible conjugates; varying the pH from 5.8 to 8.5; incubation at 25 and 37° (Singh, 1973). The lack of reactivity could be due to the epoxide moiety being trisubstituted since Fjellstedt *et al.* (1973) show that monosubstituted epoxides conjugate with GSH.

Enzymatic Oxidation of the Ethyl Epoxide. The ethyl epoxide is converted to 12 tentatively identified metabolites on incubation with rat, mouse, or rabbit liver microsomes in the presence of NADPH (Table II). The tlc pattern with rat microsomes (Figure 5) is typical of that with mouse or rabbit microsomes except for the unidentified metabolites (Singh, 1973). The metabolized portion that has undergone attack at the epoxide moiety *vs.* the ethyl group *vs.* the 2,3-double bond varies, respectively, with

Table I. Effect of NADPH Level and Protein Concentration on Metabolism of the [*phenyl*-^{14}C]- or [*phenyl*-^{3}H]Ethyl Epoxide by the Microsomal or Soluble Fraction of Rat and Mouse Liver

Enzyme source	Protein, mg	NADPH, μM	Amount of individual metabolite, % of recovered radioact.								Total recov., % of initial radioact.
			Epoxide				Diol				
			Ethyl	Aceto-	Hydroxyethyl	Ethyl diepoxide	Ethyl-	Aceto-	Hydroxyethyl-	Unknowns	
Effect of NADPH with Rat Soluble Fraction											
Rat	8H[a]	0	100				0.4			<0.1	74
	8	0	13				84	1	0.4	2	58
	8	0.5	12				70	5	5	8	80
Effect of NADPH Level with Rat and Mouse Microsomes											
Rat	2	0	87	<0.1	<0.1	<0.1	13	<0.1	<0.1	0.3	
	2	0.1	80	<0.1	<0.1	<0.1	14	<0.1	<0.1	6	
	2	0.3	56	3	12	7	10	2	2	8	
	2	1.0	17	11	19	7	6	7	5	28	
Mouse	2	0	49	<0.1	<0.1	<0.1	47	<0.1	<0.1	4	
	2	0.1	42	<0.1	3	<0.1	36	2	11	6	
	2	0.3	25	1	7	2	21	5	27	12	
	2	1.0	2	3	5	0.5	3	19	37	30	
Effect of Protein Concentration with Rat and Mouse Microsomes											
Rat	2H[a]	0.5	100				0.3			<0.1	69
	0.2	0.5	40	3	33		10	2	0.9	11	62
	1	0.5	63	3	20		7	1	1	5	65
	4	0.5	73	2	10		8	0.8	1	5	65
Mouse	0.2	0.5	17	4	37		6	4	5	27	56
	1	0.5	27	4	30		7	5	8	19	58
	4	0.5	24	1	6		25	5	26	13	74

[a] Enzyme denatured by heat (100°, 10 min).

Table II. Metabolites of the [*phenyl*-^{3}H]Ethyl Epoxide Formed by the Rat, Mouse, and Rabbit Liver Microsomal Systems Fortified with NADPH

Compound	Amt of individual metabolite, % of recovered radioact.[a]		
	Rat	Mouse	Rabbit
Epoxides			
Ethyl	63	34	20
α-Hydroxyethyl	11	10	10
β-Hydroxyethyl	1	0.6	
Aceto	3	0.7	4
Diepoxides			
Ethyl	6	2	2
α-Hydroxyethyl	1	2	
Aceto			0.5
Diols			
Ethyl-	4	18	27
α-Hydroxyethyl-	1	17	22
Aceto-	2	3	6
Cyclic products			
cis-Ethyltetrahydrofurandiol	0.9	3	2
trans-Ethyltetrahydrofurandiol	2	3	3
Ethyl bicyclic ethers I and II	0.5		
Unidentified metabolites[b]	5	7	4

[a] The total recoveries of the initial radioactivity were 62, 72, and 63%, respectively, for the rat, mouse, and rabbit liver microsomal systems. Comparable studies with [*geranyl*-^{3}H]ethyl epoxide showed similar qualitative results and only minor differences in quantitative values. [b] About 10% of this amount is retained at the origin on tlc.

the species as follows: rat, 1.0:1.8:1.0; mouse, 1.0:0.8:0.2; rabbit, 1.0:0.7:0.1. Rat microsomes are relatively less active in ethyl epoxide metabolism but they are more selective than mouse or rabbit microsomes in attacking the ethyl group and 2,3-double bond.

Whereas identification of the β-hydroxyethyl epoxide, acetodiepoxide and the ethyl bicyclic ethers I and II is based only on cochromatography, the identity of the other mouse and rat metabolites was confirmed by converting them to several types of derivatives and appropriate cochromatography in each case (Gill *et al.*, 1972; Singh, 1973). Compounds not detected as metabolites in any case include the acetotetrahydrofurandiols and the ethyl- and acetotetraols.

The mouse liver microsome–NADPH system forms metabolites with either the [*phenyl*-^{3}H]- or [*geranyl*-^{3}H]ethyl epoxide that are 87% extractable as neutral products into ether; only a small proportion of the radioactivity appears in the acid (4%) or aqueous (5%) fractions or is unaccounted for (4%). There is about 15% loss of neutral metabolites during analysis since only 72% of the radioactivity is accounted for after tlc (Table II). There is little difference in the metabolite pattern as detected with either the ring- or geranyl-labeled preparations (Table II) indicating that ether cleavage does not occur at the low levels of NADPH used.

The ratio of the major microsomal metabolites of the ethyl epoxide varies with the species, NADPH levels, and protein concentrations (Table I). No metabolism occurs with heat-treated microsomes. Metabolism shifts from almost only epoxide hydration in the absence of NADPH to an increasing importance of benzylic oxidation and formation of other oxidized metabolites along with epoxide hydration as the level of cofactor is increased (Table I). Comparable studies (Singh, 1973) with mouse or rat liver microsomes and other cofactors (NADH, $NADP^+$, NAD^+, GSH, flavine adenine dinucleotide) indicate that only NADPH is appropriate for extensive conversion to metabolites formed by oxidation. The amount of ethyldiol is reduced by addition of NADPH when high levels (Table I) or long incubation times (Figure 4) are involved, due to competing oxidation reactions when the cofactor is present. Increasing the NADPH concentration gives larger amounts of polar unidentified metabolites; whether some

of these contain cleaved ether groups is not known. At low microsome levels benzylic hydroxylation is the major route of metabolism with epoxide hydration occurring to a lesser extent. Benzylic hydroxylation is decreased on increasing the rat microsome protein concentration. The reduced ethyl epoxide metabolism at higher protein concentrations is possibly due to binding at nonenzymatic sites or to increased destruction of NADPH by side reactions. Increasing the mouse microsome concentration increases epoxide hydration with only a slight decrease in benzylic hydroxylation. Thus, at low protein concentrations the benzylic hydroxylation takes place without significant hydration while at increased protein levels benzylic hydroxylation takes place either before or after epoxide hydration. With both mouse and rat liver microsomes there is a decrease in formation of other metabolites at high protein concentrations.

The extent of oxidative metabolism of the ethyl epoxide by mouse tissue fractions incubated with NADPH is less with the cell nuclei and debris fractions of liver and kidney, the mitochondrial fraction of liver and kidney, the microsomal fraction of kidney, and the microsomal plus soluble fraction of testis than with the liver microsomal fraction, but in each case the pattern of metabolites is very similar or the same (Singh, 1973).

Metabolism of the Ethyldiol by the Microsome or Soluble Fraction of Mouse Liver. Metabolism of the *trans*-ethyldiol by microsomes, which requires both fresh enzyme and NADPH, gives the α-hydroxyethyldiol (14%), acetodiol (1%), *cis*- and *trans*-ethyltetrahydrofurandiols (2 and 3%, respectively), ethyltetrahydropyrandiol II (0.7%), ethyl α-hydroxy ketone (0.4%), and 12 minor unidentified metabolites (6% total) both more and less polar than the ethyldiol. A metabolite (0.7%) chromatographing just below the α-hydroxyethyldiol is probably the β-hydroxyethyldiol but no authentic standard was available. No ethyltetraol was found even though epoxidation occurs at the 2,3-double bond as evidenced by the formation of the ethyltetrahydrofurandiols; thus, 2,3-hydration is slow with microsomes compared to internal nucleophilic cyclization (Hammock *et al.*, 1974a).

The *cis*-ethyldiol is not metabolized by mouse liver microsomes alone but it is more extensively metabolized than the *trans*-ethyldiol by the microsome–NADPH system giving major products having the R_f values anticipated for the *cis*-α-hydroxyethyl- and acetodiols along with numerous other unidentified metabolites (Singh, 1973).

The *trans*-ethyldiol also gives the α-hydroxyethyldiol, acetodiol, and ethyltetrahydrofurandiols with mouse liver soluble fraction and their formation requires $NADP^+$ or NADPH fortification, NAD^+ or NADH being inappropriate cofactors (Singh, 1973). The active cofactor is probably NADPH, the $NADP^+$ undergoing reduction by components in the soluble fraction. One of the metabolites, with a much higher R_f than the ethyldiol, is neither the ethyl α-hydroxy ketone, the ethylaldehyde, nor the tetrahydrofuran alcohol formed by 3,6-cyclization (compound **35A** of Hammock *et al.*, 1974a).

Metabolism of the Ethyldiene in the Rat and Mouse Liver Microsome–NADPH Systems. The ethyldiene undergoes no metabolism when incubated with either heat-treated or fresh rat or mouse liver microsomes. On addition of NADPH, the rat microsomal fraction metabolizes 9% of the ethyldiene to give the acetodiene (1%), α-hydroxyethyldiene (2%), ethyl epoxide (2%), α-hydroxyethyl epoxide (0.4%), ethyldiol (0.5%), ethylphenol (0.6%), acetophenol (0.4%), and four unidentified metabolites (2%). The mouse microsome–NADPH system gives each of these metabolites in even larger amounts except acetophenol which was not detected. The ethyl epoxide is formed enzymatically and is not an artifact; it is not formed in the absence of NADPH or with heated microsomes; addition of butylated hydroxytoluene to the peroxide-free ether prevented epoxide formation during extraction and chromatography. Attempts to accumulate the ethyl epoxide as a metabolite in both rat and mouse microsome–NADPH systems were unsuccessful, probably due to cleavage of the ethyl epoxide by epoxide hydratases or competing reactions. The ether cleavage encountered with the ethyldiene is unusual, not being found on metabolism of the ethyl epoxide, ethyl diepoxide, or ethyldiol.

Metabolism of the Ethyl Diepoxide by Rat Liver Microsome Systems. The ethyl diepoxide with fresh but not heat-treated microsomes yields the ethyltetrahydrofurandiols and ethyltetrahydropyrandiols I and II, with a larger tetrahydropyran-:tetrahydrofurandiol ratio than is obtained on aqueous acid treatment (Hammock *et al.*, 1974a); this suggests that different cyclization mechanisms are involved in the two cases. Other metabolites tentatively identified by cochromatography are the ethyl bicyclic ethers I and II and the ethyloxepanediol. Fortification with NADPH gives the α-hydroxyethyl diepoxide as an additional metabolite (Singh, 1973).

Metabolism of the Acetoepoxide, α-Hydroxyethyl Epoxide, Acetodiol, Ethyltetrahydrofurandiols, and Nitroepoxide by the Rat Liver Microsome–NADPH System. The acetoepoxide gives only one major metabolite, the acetodiol, in about 20% yield (Singh, 1973). The α-hydroxyethyl epoxide gives the α-hydroxyethyl- and acetodiols as the major products plus the acetoepoxide and four other minor metabolites (Singh, 1973). The acetodiol and the ethyltetrahydrofurandiols are not metabolized indicating that these are possibly terminal metabolites in the rat liver microsome–NADPH system (Singh, 1973). Metabolism of the nitroepoxide in the absence of NADPH gives only one metabolite and it chromatographs as anticipated for the nitrodiol; on addition of NADPH there is much more extensive metabolism yielding photolabile products suggesting that the nitro group undergoes modification (Singh, 1973).

Algae. The growth of algal cultures at 27° is delayed at the following juvenoid concentrations (parts per million): *Chlorella*, 0.1 for ethyldiene, 0.2 for ethyl epoxide, and 10 for ethyldiol; *Chlamydomonas*, 1 for ethyldiene and 10 for ethyl epoxide, ethyldiol, and two aliphatic diene esters (Altosid and Altozar Insect Growth Regulators; Henrick *et al.*, 1973). Considerably higher juvenoid concentrations (2–100 fold) are needed to block growth. Thus, these compounds delay the time before algal growth enters the log phase but not the growth rate within the log phase (Hammock, 1973). The potency of the ethyl epoxide and ethyldiene is increased about tenfold when *Chlamydomonas* cultures are held at 5° for 48 hr before inoculation into the treated medium.

Table III shows the products formed when the ethyldiene, ethyl epoxide, and ethyldiol are incubated in light with *Chlamydomonas* or *Chlorella* cultures or chlorophyll. The products are formed in lower amounts or not at all in the absence of light. The metabolites are tentatively identified by cochromatography with the following additional evidence supporting their structures: the ethyl- and α-hydroxyethyldiols give appropriate BBA derivatives and the ethyl- and acetophenols are base-extractable compounds. There are also large amounts of acidic products formed under many of the incubation conditions (Hammock, 1973).

The ethyldiene and ethyl epoxide are over 90% degraded by *Chlamydomonas* in the dark within 12 hr while little degradation of the ethyldiol occurs even after 12 days. *Chlamydomonas* in light yields two unidentified metabolites from the ethyldiene or ethyl epoxide, both of which have higher R_f values than the ethyl epoxide; all of the other unidentified metabolites of either compound chromatograph between the ethyl epoxide and ethyldiol. After 48 hr of incubation with *Chlamydomonas* the degradation

Table III. Products Formed on Incubation of the Ethyldiene, Ethyl Epoxide, and Ethyldiol with *Chlamydomonas* or *Chlorella* Cultures or Chlorophyll in Light

	Product amount[a] in different incubation media								
	Chlamydomonas			*Chlorella*			Chlorophyll		
Compound	Diene	Epoxide	Diol	Diene	Epoxide	Diol	Diene	Epoxide	Diol
Dienes									
Ethyl-	*b*			*b*			*b*		
α-Hydroxyethyl-	+								
Aceto-	+								
Epoxide									
Ethyl	+	*b*			*b*			*b*	
Diepoxide									
Ethyl	+	+					++	++	
Diols									
Ethyl-	++	+++	*b*	+	+	*b*	+	+	*b*
α-Hydroxyethyl-	+	+	+		+	+++			+
Tetraol									
Ethyl			+						
Tetrahydrofurandiols									
Ethyl	+	+	+			+			+
Phenols									
Ethyl-	+	+				−			++
Aceto-	+	+	+	+		−	++	++	+

[a] Amounts are designated as follows: +++, over 90% of the organosoluble radioactivity; ++, major product; +, minor product; −, absent. [b] Starting material.

products are similar starting with any one of the ethyldiene, ethyl epoxide, or ethyldiol, major products being the ethyl- and α-hydroxyethyldiols and one chromatographing between the α-hydroxyethyldiol and the ethyltetraol; after 35 days, acetophenol is the major product and a product cochromatographing with the ethyltetraol is found. *Chlorella* degrades all three compounds faster than *Chlamydomonas*, particularly the ethyldiene which is completely degraded within 24 hr, almost entirely to the α-hydroxyethyldiol. With *Chlorella* in light the ethyldiene is completely metabolized within 17 hr but 30% of the ethyl epoxide remains intact after 48 hr, its major metabolites being the ethyl- and α-hydroxyethyldiols.

Incubation with chlorophyll gives the same or similar products as incubations with culture medium alone or sonication-killed algae. The ethyldiene is much more rapidly degraded than the ethyl epoxide while the the ethyldiol is very stable. Major degradation products of the ethyldiene and ethyl epoxide are the ethyl diepoxide and ethylphenol at 24 hr and acetophenol at 48 hr (Table III) (Hammock, 1973).

Photodecomposition. The rate of radioactivity loss on exposure of the [*phenyl*-^{3}H]ethyl epoxide on glass surfaces to sunlight or uv irradiation is biphasic, the more rapid initial phase probably resulting from volatilization of the ethyl epoxide and the subsequent slower phase from loss of less volatile and more polar photoproducts. The recovery of intact [*geranyl*-^{3}H]ethyl epoxide on silica gel plates is as follows at 4 and 38 hr, respectively: 98 and 96% in the dark; 92 and 66% with sunlight or sunlamp irradiation; 50 and 15% with uv irradiation. Most of the photoproducts from exposure to sunlight or sunlamp irradiation move free from the origin on tlc while those from uv irradiation mostly remain at the origin (Singh, 1973).

In a study with 22 candidate photosensitizers, no general correlation was evident between their triplet energy values (39–74 kcal/mol) or structure and the degree of photosensitization of the [*geranyl*-^{3}H]ethyl epoxide. Dyes generally show no sensitizing activity. Sensitizers catalyzing extensive ethyl epoxide degradation (*i.e.*, xanthen-9-one, anthracene, and 7*H*-benz(de)anthracen-9-one) resulted in low recoveries of total radioactivity (46–60%) possibly due to tritium loss on ether cleavage or C-1 hydroperoxide formation. The extent of photoalteration of the ethyl epoxide on exposure to sunlight for 8 hr increased with the amount of xanthen-9-one up to the point when a xanthen-9-one:ethyl epoxide ratio of 0.5 was reached (Singh, 1973).

The ethyl epoxide is stable on silica gel chromatoplates or in water when held 8 hr in the dark, giving only trace amounts of ethyldiol in water. However, the ethyl epoxide when exposed to sunlight on silica gel undergoes extensive degradation yielding products generated by reactions at the epoxide moiety, the 2,3-double bond, and the ether linkage (Table IV). Addition of xanthen-9-one greatly reduces the level of ethyl diepoxide and the major unknown I but it increases the extent of benzylic oxidation to the acetoepoxide, α-hydroxyethyl epoxide, and acetophenol. The ethyl epoxide is relatively resistant to photodecomposition in aqueous solution, the ethyldiol being the major product with small amounts of other materials including the ethyl- and acetophenols (Figure 5, Table IV). The structure of the [*phenyl*-^{3}H]ethyl diepoxide photoproduct was verified by treating it with acid and then cochromatographing with the products generated on similar treatment of authentic unlabeled ethyl diepoxide. Derivatization studies support the structures of other photoproducts as follows: the acetoepoxide forms the acetodiol with aqueous acid, the α-hydroxyethyl epoxide with $NaBH_4$, and a semicarbazone with semicarbazide; the α-hydroxyethyl epoxide gives the α-hydroxyethyldiol with aqueous acid, the acetoepoxide with lead tetraacetate, and does not react with $NaBH_4$. The following photoproducts isolated from a large scale photodecomposition on silica gel were identified as indicated: ethyl epoxide [67%; pmr, infrared (ir)]; ethyldiol [8%; pmr, ir, mass spectroscopy]; ethylphenol (7%; pmr, ir). Functional group tests (OsO_4, BBA, Ac_2O) and chromatographic characteristics suggest that the major unknown I is a secondary alcohol with no sites of olefinic unsaturation. Major unknown II appears to be an acidic compound. The cis and trans isomers of the ethyl epoxide undergo hydration to the ethyldiol at similar rates in aqueous solutions exposed to sunlight and the resulting *cis*- and *trans*-ethyldiols are also of similar stability.

The mixture of photoproducts formed on exposure of the ethyl epoxide to sunlight on silica gel for various time periods up to 32 hr is reduced by 100- to 1000-fold in morphogenetic activity (*Tenebrio* assay), confirming the results from assaying individual identified photoproducts of which only the acetoepoxide shows significant activity (Hammock *et al.*, 1974a).

Table IV. Photoproducts Formed on Exposure of Phenyl-Labeled Preparations of the Ethyldiene, Ethyl Epoxide, Ethyl Diepoxide, or Ethyldiol on Silica Gel or in Water to Sunlight for 4 hr

	Amount of individual product, % of recovered radioactivity							
	Silica gel, 30 nmol/cm²					Water, 0.5 ppm		
Compound[a]	[³H]Ethyl-diene[b]	[³H]Ethyl epoxide[b]	[¹⁴C]Ethyl diepoxide	[¹⁴C]Ethyl-diol	[³H]Ethyl epoxide sensitized with xanthen-9-one[c]	[³H]Ethyl-diene	[¹⁴C]Ethyl epoxide	[¹⁴C]Ethyl-diol
Dienes								
Ethyl-	17					9		
Epoxides								
Ethyl	10	74			22	11	81	
α-Hydroxyethyl					12		1	
Aceto-					17		0.6	
Diepoxides								
Ethyl	2	3	37			6	1	
α-Hydroxyethyl			(0.8)					
Aceto-			(0.8)					
Acyclic alcohols								
Diols								
Ethyl-		0.5		55		2	8	94
α-Hydroxyethyl-							(1)	0.8
Aceto-							(0.2)	0.7
Ethyl-6-hydrox-2-ene	4							
Ethyl α-hydroxy ketone		(0.6)		(0.9)				
Ethyl-6-hydroxy-2,7-diene						5		
Cyclic products								
cis-Ethyltetrahydro-furandiol		0.3	1	0.9	(1)	(0.9)	0.5	0.7
trans-Ethyltetrahydro-furandiol		0.2	1	1	(2)	(1)	0.6	0.5
Ethyltetrahydropyran-diol I		0.7	16			(1.2)		
Ethyltetrahydropyran-diol II		0.4	10			(2)		
Ethyloxepanediol			7					
Ethyl bicyclic ethers I and II		1	2					
Phenols								
Ethyl		0.4		1		4	1	
Aceto		(0.3)		(0.7)	4	1	0.5	0.3
Unknowns[d]								
Major I[e]	28	14	5	16	6	18	2	0.8
Major II[e]	17	1	4	5	5	4	1	0.7
Others	18	1	7	14	16	34	0.6	0.9
Origin	4	2	8	5	15	1	0.2	0.5
Total recovery, % of initial radioact.	79	89	91	83	54	71	95	99.5

[a] The use of parentheses in reporting the quantitative data indicates that these products cochromatograph with authentic compounds of the indicated structure but the photoproduct was in small amount or the tlc system did not adequately resolve this material from others that interfere with suitable cochromatography. [b] Comparable studies with the geranyl-³H-labeled compounds showed similar qualitative results and only minor differences in the quantitative values. [c] Xanthen-9-one (15 nmol/cm²) was applied before the ethyl epoxide. No products appear in the chromatographic region of acetophenol when the geranyl-³H-labeled compound is used; however, this ³H-labeled preparation yields two photoproducts in 3.5 and 5% yields with chromatographic behavior similar to but not identical with that of the ethyldiol (Singh, 1973). [d] The chromatographic positions and amounts of individual unknowns are given by Singh (1973). [e] The designations for each unknown refer only to the particular compound and conditions of irradiation and in no way imply that they are structurally similar or identical when different compounds or conditions are used.

The ethyldiene on silica gel is slowly converted to the ethyl epoxide and to major unknown I in the dark but the amount of ethyl epoxide formed is increased 12-fold and of unknown I by 3-fold on exposure to sunlight for 6 hr (Singh, 1973). The ethyl substituent of the ethylphenoxy moiety and the ether linkage are resistant to photooxidation or cleavage. The photoproducts are identified only by cochromatography except for the ethyl epoxide which was converted to the ethyldiol with aqueous acid. The major unknown I chromatographs as anticipated for a keto compound when compared to the ethyl-6-hydrox-2-ene; if this photoproduct is the ethyl-6-ket-2-ene from oxidation of the 6-hydroxy compound it is comparable to products formed on photooxidation of trisubstituted olefins (Chen and Casida, 1969) and rearrangement of trisubstituted epoxides (Joshi *et al.*, 1971) on solid matrices. In aqueous medium, the ethyldiene gives essentially only the ethyl epoxide in the dark but in sunlight it undergoes extensive reactions of many types (Table IV); additional products not detected are the α-hydroxyethyl- and acetodienes, the aceto-6-hydroxy-2,7-diene, and 4-(4′-acetophenoxy)-2-methyl-2-butenoic acid (Singh, 1973).

The ethyl diepoxide is converted mainly to cyclic products when exposed on silica gel to sunlight (Table IV). Several isomers of the tetrahydropyrandiols may be present since the standards for cochromatography were themselves mixtures of isomers not resolved by tlc. The structures of the individual isomers of the ethyloxepanediol

and the ethyl bicyclic ethers I and II are not assigned (Hammock *et al.*, 1974a). Cochromatography shows the absence of the ethyl- and acetotetraols. The ethyltetrahydrofurandiols are produced in very small amounts as compared to their formation on treating the ethyl diepoxide with acid (Hammock *et al.*, 1974a). The major unknowns (I and II) are also formed as minor products on acid treatment of the ethyl diepoxide. Benzylic oxidation is a minor route based on the identified compounds (Table IV) and the anticipated chromatographic positions of the α-hydroxyethyl and aceto derivatives of the cyclic products.

The ethyldiol is more stable than the other compounds on silica gel or in aqueous solutions when held in the dark. Identifications of the ethyl α-hydroxy ketone and acetophenol as photoproducts are tentative. Unknown I from the ethyldiol and ethyl epoxide is the same compound based on extensive chromatographic comparisons with standards of somewhat similar chromatographic properties and the reactions noted with Ac_2O- and OsO_4-pyridine.

DISCUSSION

The pathways for metabolism in mammals and algae and for photoalteration of the ethyldiene, ethyl epoxide, ethyl diepoxide, and ethyldiol are given in Figure 1. The major pathways involve 6,7-epoxidation and -hydration, 2,3-epoxidation and -hydration with or without cyclization, oxidation of the ethyl side chain, to a greater extent at the α than at the β position, and ether cleavage. Epoxidation of the ethyldiene to the epoxide is the only step resulting in a great increase in morphogenetic activity (Hammock *et al.*, 1974a).

In living rats and mice, the phenyl-labeled ethyl epoxide is rapidly metabolized and the metabolites are excreted without persistent storage in tissues. Orally administered ethyl epoxide is likely to undergo rapid acid-catalyzed hydration to the ethyldiol in the stomach. Conjugation is also important in ethyl epoxide metabolism in rats and mice, as well as insects (Hammock *et al.*, 1974b). A major portion of the ethyl epoxide dose undergoes ether cleavage in rats (Hoffman *et al.*, 1973) and mice whereas under *in vitro* conditions ether cleavage does not occur due possibly to competing reactions. Ether cleavage *in vivo* is probably initiated by oxidation at the activated *O*-methylene position. O-Dealkylation might be further facilitated by preliminary oxidation of the ethyl to the aceto, carboxy, and carboxymethyl groups. However, O-dealkylation in mice can occur prior to oxidation of the side chain since ethylphenol is excreted.

The ethyl group of the ethyl epoxide is extensively metabolized in living rats yielding carboxymethyl, carboxy, and 4-hydroxyphenyl derivatives (Hoffman *et al.*, 1973). The present study with mice confirms the presence of hydroxybenzoic and hydroxyphenylacetic acids as *in vivo* urinary metabolites of the ethyl epoxide but it did not detect the hydroxydiene and -diol, carboxytetraol, and ethyl-2,3-diol-6,7-epoxide reported as rat metabolites by Hoffman *et al.* (1973). The urinary and fecal metabolites of mice do not include any one of the carboxymethyldiene and -diol, the carboxydiene and -diol, and the hydroxydiene, hydroxy epoxide, and hydroxydiol, compounds that would result from extensive oxidation or cleavage of the ethyl moiety while retaining the ether linkage. In analogy with metabolic studies on other alkylbenzenes (reviewed by Singh, 1973), the complete scission of the ethyl group in the ethyl epoxide is an unanticipated reaction. However, the differences in extent of metabolism of the ethyl epoxide at the ethylphenyl moiety in the present study and that of Hoffman *et al.* (1973) may also result from one or more procedural variations between the two studies as follows: mice *vs.* rats; oral administration of a 1 mg/kg dose in ethanol *vs.* ip administration of a 1800 mg/kg dose in corn oil. No metabolites having a 6,7-double bond were detected in the present study; thus ethyl epoxide fecal metabolites of this type reported from *in vivo* studies by Hoffman *et al.* (1973) possibly arose, as they suggest, from metabolism of the ethyldiene present as a trace contaminant in the original ethyl epoxide preparation. Identification by mass spectroscopy of the ethyl-2,3-diol-6,7-epoxide as an ethyl epoxide metabolite (Hoffman *et al.*, 1973) is probably incorrect since this epoxydiol rapidly cyclizes giving the ethyltetrahydrofurandiols (Hammock *et al.*, 1974a) and identical mass spectral data are reported for the ethyltetrahydrofurandiols (Hammock, 1973) and the proposed ethyldiol epoxide (Hoffman *et al.*, 1973).

The ethyl substituent is preferentially oxidized by the microsome–NADPH system at the activated benzylic methylene group with subsequent oxidation of the α-hydroxyethyl compound, catalyzed more effectively by soluble than microsomal enzymes, to the aceto derivative. Only small amounts of the β-hydroxyethyl compounds are found and the acids resulting from their further oxidation are not detected.

The ethyldiene is epoxidized to the ethyl epoxide in all microsome–NADPH systems examined. In the rat system, the ethyldiene is poorly metabolized relative to the ethyl epoxide and ethyldiol, whereas in house fly (Hammock *et al.*, 1974b) and mouse systems there is extensive modification of the ethyldiene. Epoxidation of the 2,3-double bond, which occurs to a greater extent with the ethyl epoxide than with the ethyldiol, converts the epoxide to the diepoxide and both the ethyl epoxide and ethyldiol to the ethyltetrahydrofurandiols and other cyclic ethers which probably arise *via* the 2,3-epoxy-6,7-diol (Hammock *et al.*, 1974a).

The ethyl epoxide undergoes rapid enzyme-catalyzed hydration to the ethyldiol. The epoxide hydratase activity of rat liver acting on the ethyl epoxide can be compared with that of guinea pig liver acting on styrene oxide (Oesch, 1973; Oesch *et al.*, 1971a,b) as follows: similar tissue distribution; the most active subcellular fraction is the soluble with the ethyl epoxide and the microsomes with styrene oxide; compounds that inhibit hydration of styrene oxide do not inhibit ethyl epoxide hydration by microsomes. These differences could result from one or more of the following: species specificity; the presence of more than one epoxide hydratase in the microsomes (Oesch, 1973; Oesch *et al.*, 1971a); the involvement of different hydratases or hydration mechanisms with highly lipophilic trisubstituted epoxides (the ethyl epoxide) than with monosubstituted epoxides (styrene oxide). Additional species and substrate specificity studies are necessary to clarify these speculations.

Enzymatic hydration of the ethyl diepoxide gives rise to a number of cyclic products similar to those formed on aqueous acid treatment, indicating that an electrophilic site on the enzyme catalyzes formation of an epoxydiol which subsequently undergoes intramolecular cyclization. No tetraols are detected as ethyl epoxide or diepoxide metabolites in either house fly (Hammock *et al.*, 1974b) or rat liver microsomes, yet the acetotetraol is an *in vivo* metabolite of the ethyl epoxide in rats (Hoffman *et al.*, 1973) and mice. Thus, the ethyl diepoxide is not a likely intermediate in formation of the acetotetraol. Tetraol formation may proceed *via* the 2,3-epoxy-6,7-diol involving very rapid hydration of the 2,3-epoxide compared to the time required for cyclization giving the ethyltetrahydrofurandiols; alternatively, the ethyl epoxide undergoes 2,3-epoxidation and 2,3- and 6.7-hydration, in an undetermined sequence, while remaining bound at a position proximal to both oxidase and hydratase sites. Another possibility is that in some cases of glycol formation at a double bond an epoxide intermediate is not involved.

Inhibition of algal growth is unlikely to limit the use of phenyl geranyl ether juvenoids in control of mosquito lar-

vae since they block insect development at much lower levels. However, there are conceivable situations of low populations and slow growth rates where application of an inappropriate juvenoid could lead to a detrimental shift in the composition of a phytoplankton community.

The ethyldiene and ethyl epoxide are both rapidly degraded by algae giving a variety of products including ethyl- and acetophenols. *Chlamydomonas* carry out rapid oxidation and then hydration of the 6,7-double bond. The 2,3-double bond is relatively resistant to oxidation as evidenced by the low levels of the ethyl diepoxide and ethyltetrahydrofurandiols formed.

One photoproduct, the ethyl-6-hydrox-2-ene, may undergo oxidation to the corresponding keto analog since comparable ketones form on photooxidation of trisubstituted olefins (pyrethroids) and isomerization of trisubstituted oxiranes (citronellol epoxide) (Chen and Casida, 1969; Joshi *et al.*, 1971). The ethyl-6-hydroxy-2,7-diene, a photoproduct in aqueous medium, is formed in good yield on Rose Bengal photosensitized oxidation of the ethyldiene followed by chemical reduction (Hammock, 1973); the corresponding allylic hydroperoxides are found on irradiation of acyclic monoterpenes (Schenck *et al.*, 1962). Epoxides also give allylic alcohols by isomerization on alumina (Dunphy, 1970; Joshi *et al.*, 1971). However, on exposure of aqueous solutions to sunlight only the ethyldiene and not the ethyl epoxide give the allylic alcohol so epoxidation is not an intermediate step in this photochemical conversion.

The ethyl epoxide undergoes significant photoepoxidation to the ethyl diepoxide. A similar epoxidation occurs with the C_{18}-juvenile hormone on uv exposure (Pawson *et al.*, 1972). The cyclic diols and ethers other than the ethyltetrahydrofurandiols probably arise by photoepoxidation of the ethyl epoxide and then hydration of the ethyl diepoxide rather than 2,3-epoxidation of the ethyldiol since they form in larger amounts from the diepoxide and are not photoproducts of the ethyldiol.

Photodecomposition of the ethyl epoxide and particularly the ethyl diepoxide gives larger amounts of the ethyltetrahydropyrandiols I and II than of the ethyltetrahydrofurandiols; in contrast, the ethyltetrahydrofurandiols predominate over the ethyltetrahydropyrandiols on enzymatic degradation of the ethyl epoxide, ethyl diepoxide and ethyldiol and are in even greater excess upon aqueous acid cleavage of the ethyl diepoxide. The ethyltetrahydrofurandiols are the only products on oxidation of the ethyldiol in organic media (Hammock *et al.*, 1974a). Thus, the relative amounts of the cyclic diols appear to be determined by the nature of the medium.

Ethyl epoxide hydration to the ethyldiol is the major route of photoalteration in aqueous media; however, on silica gel oxidation of the 2,3-double bond also occurs to yield an unidentified cyclic monohydroxy derivative. In aqueous media, the ethyl epoxide and ethyldiol give the *cis*- and *trans*-ethyltetrahydrofurandiols as the only identified cyclic products, the ethyldiol being a likely intermediate in conversion of the ethyl epoxide to the ethyltetrahydrofurandiols.

The ethyl epoxide and ethyldiol in aqueous media and the ethyl epoxide on silica gel when sensitized with xanthen-9-one undergo benzylic oxidation. The α-hydroxyethyl compound is presumably the intermediate in formation of the aceto compound. Photochemical ether cleavage forming phenols, which occurs with many of the test compounds and conditions, results either by photolysis of the carbon–oxygen bond or photooxidation at the activated α-carbon giving the unstable α-hydroperoxide as an intermediate.

The ethyl epoxide, ethyldiene, ethyl diepoxide, and ethyldiol undergo rapid and extensive modifications with loss of morphogenetic activity in each of the biological and photochemical systems examined. This limited persistence is advantageous in minimizing environmental pollution but detrimental in the practical use of juvenoids in pest insect control. The groupings that are most labile to degradation are the ethylphenyl and epoxide moieties, the 2,3-double bond, and the ether linkage. Substitution of any of these groupings with others more resistant to metabolism and photodecomposition might yield compounds of increased effectiveness. Possible modifications include the use of alkoxides instead of epoxides (Sarmiento *et al.*, 1973), saturation of the 2,3-double bond, and replacement of the ethyl moiety with an appropriate halogen or other group. In any case, effective pest insect control with juvenoids requires their application at a time and in a manner to contact sensitive stages in the life cycle of the pest.

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