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Metabolic O-Dealkylation of 1-(4′-Ethylphenoxy)-3,7-dimethyl-7-methoxy or ethoxy-*trans*-2-octene, Potent Juvenoids[1]

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1-(4′-Ethylphenoxy)-3,7-dimethyl-7-methoxy or ethoxy-*trans*-2-octene (the ethyl-methoxide and ethyl-ethoxide) are more stable in some biological systems and less stable in others than 1-(4′-ethylphenoxy)-3,7-dimethyl-6,7-epoxy-*trans*-2-octene (the ethyl-epoxide). In housefly adults and mealworm pupae the persistence increases in the order of the ethyl-epoxide, -methoxide and -ethoxide but with adult stable flies the epoxide is of intermediate stability. The alkoxides are metabolized in living insects and microsomal oxidase systems of houseflies and mouse liver mainly by *O*-dealkylation, at a higher rate for the methoxide than the ethoxide, but benzylic oxidation of the ethyl group also occurs and is more important in the degradation of the ethyl-epoxide than the ethyl-alkoxides. The photostability on silica gel is slightly better for the ethyl-ethoxide than the -methoxide or -epoxide.

Phenyl 6,7-epoxygeranyl ethers with appropriate *para*-substituents are effective juvenoids (juvenile hormone mimics) (1–4) but their utilization in pest insect control is limited under some conditions by the instability of the epoxide moiety (5, 6). The corresponding 7-alkoxygeranyl ethers also have high morphogenetic activity and it is anticipated that under some biological and environmental conditions they will be of increased stability relative to the epoxides (6–9). This study compares the metabolic and photochemical stability of the *p*-ethylphenyl ethers derived from geraniol, 6,7-epoxygeraniol, 7-methoxygeraniol, and 7-ethoxygeraniol.

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MATERIALS AND METHODS

Chemicals. The synthesis scheme for the radioactive chemicals and unlabeled standards and the trivial names used to designate these compounds are given in Fig. 1.

Standard unlabeled compounds for cochromatographic comparison with labeled metabolites were prepared and purified as previously described (6) or by similar procedures (Fig. 1). The structures were verified on the basis of infrared, proton magnetic resonance, and chemical ionization mass spectra. The structure of the ethyl-*t*-alcohol and metabolites cochromatographing with it were verified by their reaction with OsO_4 to form the ethyl-triol and their failure to be acetylated with acetic anhydride-pyridine, while metabolites cochromatographing with the ethyl-diol were confirmed by preparation of their *n*-butylboronic acid (BBA)[4] diesters (6).

[4] Abbreviations used: BBA, *n*-butylboronic acid; PE, petroleum ether–ether; BP, benzene–propanol;

The [^{3}H]phenyl-ethyl-diene (654 mCi/mmole; see Ref. 10) was converted to the [^{3}H]ethyl-epoxide on oxidation with equimolar *m*-chloroperoxybenzoic acid (MCPBA). Alkoxymercuration was used to convert the [^{3}H]ethyl-diene to the [^{3}H]-ethyl-methoxide and -ethoxide. Thus, the [^{3}H]ethyl-diene (5.2 μmole) in either ethanol or methanol (100 μl) was added to a small ampule containing mercuric acetate (8 μmole) and a small stirring bar. After stirring (45 min) the ampule was cooled (0°C) and aq. NaOH (100 μl, 3 *N*) followed by aq. NaOH (200 μl, 3 *N*) containing $NaBH_4$ (ca. 20 μmole) were added. After 30 min the solutions were saturated with NaCl and extracted with hexane (3×, 400 μl) to recover 80% of the original radioactivity. Analysis by thin-layer chromatography (tlc) revealed a mixture of the desired alkoxide, small amounts of the corresponding dialkoxide and of starting material, and at least four other labeled compounds in each case. The alkoxides were isolated by tlc using silica gel F-254 chromatoplates developed with PE 12:1 (see below). The yields of the alkoxides with a radiochemical purity of over 99.7% were 16% for the [^{3}H]ethyl-methoxide and 30% for the [^{3}H]ethyl-ethoxide based on the radioactivity of the starting [^{3}H]ethyl-diene.

Thin-layer chromatography. Tlc was performed on precoated silica gel 60 F-254 chromatoplates (EM Laboratories, Elmsford, NY) of 0.25-mm layer thickness except when large amounts of biological extracts were spotted. In these cases precoated Quanta/gram LQF plates (Quantum Industries, Fairfield, NJ) were used. Solvent systems commonly employed are: benzene–propanol (BP) and petroleum ether–ether (PE) in various ratios, carbon tetrachloride–ether (2:1) (TE), and chloroform–methylene chloride–ethyl acetate-*n*-propanol (10:10:1:1) (CMAP). The tlc pattern for standard compounds in a combination of these solvent systems is shown in Fig. 2. Standards were visualized by their quenching of gel fluorescence, and radioactive materials were detected by autoradiography then quantified by scraping the appropriate area of the tlc plate followed by liquid scintillation counting (lsc).

Metabolism in living insects. Adult stable flies (*Stomoxys calcitrans* L.) and houseflies (*Musca domestica* L.) (SCR strain), treated topically on the ventrum of the abdomen with 1 μg of ^{3}H compound in 0.5 μl ethanol, were held in glass vials (three flies/vial) while fed on granulated sugar and water (stable flies additionally received dried mouse blood) then frozen at −20°C prior to analysis. Control flies were placed in boiling water for 2 min prior to treatment. In studies on persistence, each group of three flies was homogenized in 0.6 ml reagent grade acetonitrile then 0.2 ml of the extract was cospotted with the original standards on a 3-cm-wide band of a Quanta/gram plate and developed 6 cm with PE 10:1. The appropriate gel regions were then subjected to lsc after holding the sample in the dark for a minimum of 12 hr. Excreted radioactivity was determined by adding scintillation fluid to the vials in which the flies had been previously held. For tentative identification of metabolites by tlc cochromatography, five flies were homogenized in 1 ml of water, the water was saturated with $(NH_4)_2SO_4$, and extracted twice with 3 ml of peroxide-free ether, the ether dried (Na_2SO_4), reduced in volume under N_2, cospotted with the appropriate standards on F-254 tlc plates, and developed with the appropriate solvents (Fig. 2).

Pupae of the yellow mealworm (*Tenebrio molitor* L.) were treated similarly except that three pupae were homogenized in 3 ml of acetonitrile for quantitative studies or 3 ml of water followed by two

TE, carbon tetrachloride–ether; CMAP, chloroform–methylene chloride–ethyl acetate-*n*-propanol; tlc, thin-layer chromatography; MCPBA, *m*-chloroperoxybenzoic acid; lsc, liquid scintillation counting BSA, bovine serum albumin; NADPH-reduced nicotinamide–adenine dinucleotide phosphate.

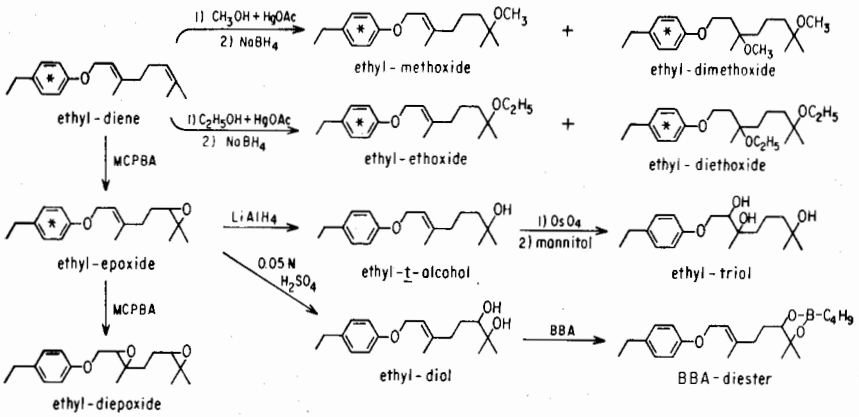


FIG. 1. *Synthesis routes for unlabeled standards, [^3H]ethyl-alkoxides and [^3H]ethyl-epoxide. The aceto series in which the p-C_2H_5-substituent is replaced with p-$CH_3C(O)$- is prepared in the same manner from the aceto-diene. The aceto series is converted to the α-hydroxyethyl or p-CH_3CHOH- series on reduction with $LiAlH_4$.*

extractions with 5 ml of ether for characterization studies.

Enzyme studies. ^3H Substrates of varying specific activities were added in ethanol (10 μl) to the housefly microsomal suspension [0.6 mg microsomal protein/ml sodium phosphate buffer, pH 7.4, 0.2 *M* containing 1% bovine serum albumin (BSA); see Ref. 11] in the presence or absence of reduced nicotinamide-adenine dinucleotide phosphate (NADPH, 0.5 μmole) followed by incubation at 30°C for 1 hr. The substrate level was 0.1 μmole unless stated otherwise. In studies with mouse liver microsomes, the enzyme concentration was 2 mg microsomal protein, the buffer was 0.1 *M* sodium phosphate at pH 7.4 containing no BSA, the substrate level was 0.1 μmole and the incubations were carried out for 30 min at 37°C. The reaction (1 ml) was terminated by addition of $(NH_4)_2SO_4$ and ether (10 ml). The ether extract was analyzed by tlc followed by lsc.

Photostability. In accordance with the

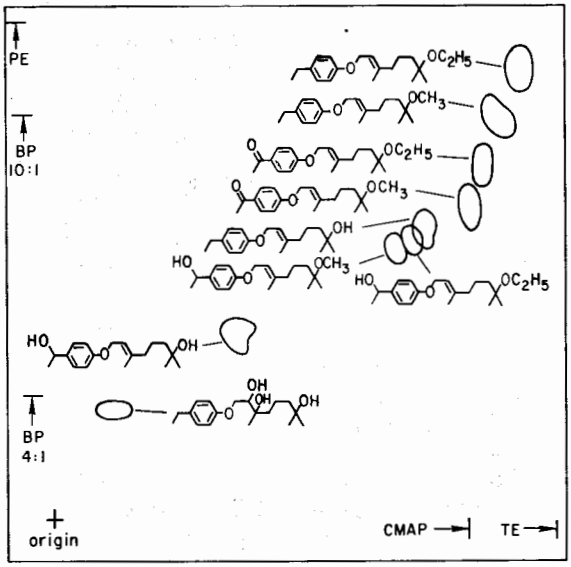


FIG. 2. *Separation of ethyl-methoxide, ethyl-ethoxide and several of their metabolites and derivatives on silica gel F-254 chromatoplates.*

TABLE 1

Persistence and Morphogenetic Activity of the Ethyl-Diene, -Epoxide, -Methoxide, and -Ethoxide in Topically Treated Insects

Species or parameter	Compound			
	Ethyl-diene	Ethyl-epoxide	Ethyl-methoxide	Ethyl-ethoxide
Half-life, hr[a]				
Housefly adult	1.5	0.5	1.0	1.5
Stable fly adult		4.5	3.5	6.5
Mealworm pupa		8.0	57	63
Morphogenetic activity in *T. molitor* assay[b]				
Effective dose for 50% response, pg/pupa	120	5	120	0.5

[a] Semilogarithmic plots of the rates of degradation or loss of the juvenoids in the three insects tested were linear at least until less than 10% of the substrate remained; the rates decreased after that point.

[b] See Ref. 6.

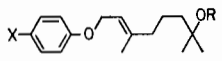
procedure of Gill *et al.* (5), a spot (40 nmole) of the ^3H compound at the origin of a silica gel F-254 chromatoplate was exposed to a sunlamp for up to 8 hr then the chromatograms were developed with BP (10:1) for 5 cm then TE (2:1) for 15 cm.

RESULTS

Metabolism in living insects. The persistence of the topically applied juvenoids is least in adult houseflies treated at 50 μg/g, intermediate in adult stable flies treated at 75 μg/g, and greatest in *Tenebrio* pupae administered a 7.5-μg/g dose (Table 1). In each case the ethyl-ethoxide persists longer than the ethyl-methoxide. The ethyl-epoxide is very rapidly metabolized in mealworms relative to the ethyl-alkoxides, as expected from their relative potencies when assayed topically for morphogenetic activity with this organism (Table 1). Heat-treated insects do not degrade the ^3H compounds. The variations in the persistence patterns are partially attributable to species differences in metabolism of the hormone mimics as described

TABLE 2

Metabolism of the Ethyl-Methoxide ($X = CH_3CH_2$, $R = CH_3$) and Ethyl-Ethoxide ($X = R = CH_3CH_2$) in Living Stable Flies and in Microsome–NADPH Systems of Houseflies and Mouse Liver



X	R	Organosoluble radioactivity recovered (%), with indicated R substituent					
		Living stable fly, 2 hr		Microsome–NADPH system			
				Housefly		Mouse	
		CH_3	CH_3CH_2	CH_3	CH_3CH_2	CH_3	CH_3CH_2
CH_3CH_2	CH_3 or C_2H_5	64	91	71	82	28	51
CH_3CH_2	H	27	6	23	7	9	14
CH_3CHOH	CH_3 or C_2H_5	<1	<1	1	2	9	
CH_3CHOH	H	1	0.2	0.4	1	6	0
$CH_3C(O)$	CH_3 or C_2H_5	<1	0.5	1	2	0	0
Unidentified		7	2	4	6	48	35

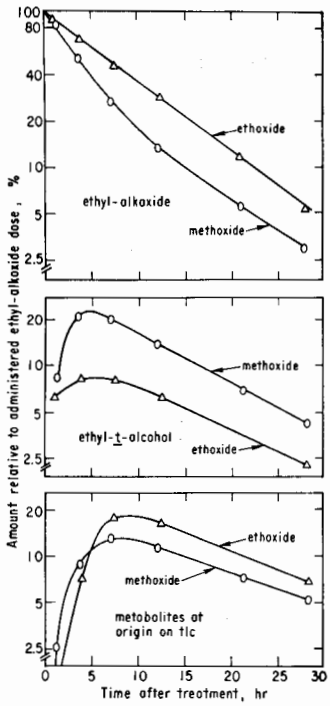


FIG. 3. *^3H Products in adult stable flies treated topically with the [^3H]ethyl-methoxide and [^3H]ethyl-ethoxide.*

below. In all cases involving houseflies and stable flies, the summation of the excreted radioactivity and that in the insects accounted for 96–101% of the administered dose.

In houseflies the only major products detected at 1–6 hr after treatment are the administered materials, only trace amounts of the ethyl-*t*-alcohol being detected from the ethyl-alkoxides and of the ethyl-diol from the ethyl-epoxide. Apparently the metabolites are rapidly excreted from the houseflies so they are not detected.

In stable flies the major metabolite of the ethyl-ethoxide and particularly the ethyl-methoxide is the ethyl-*t*-alcohol or 7-hydroxy compound formed on *O*-dealkylation (Table 2). The more rapid *O*-dealkylation of the ethyl-methoxide than of the ethyl-ethoxide is also evident from graphical considerations of their metabolism rates and the levels of the ethyl-*t*-alcohol metabolite (Fig. 3). A trace amount of benzylic oxidation is also detected with each ethyl-alkoxide (Table 2) and in addition there are unidentified metabolites that remain at the origin on tlc (Fig. 3). The ethyl-epoxide recovery under similar conditions is 74% with the following amounts of metabolites: 4.6% ethyl-diol, 1.8% α-hydroxyethyl-epoxide, 4.2% aceto-epoxide, 1.5% ethyl-diepoxide, 0.4% α-hydroxyethyl-diol, and 13.5% as 23 unidentified ether-soluble products.

In mealworms at 36 hr after treatment the ethyl-*t*-alcohol from *O*-dealkylation is present in about one-half the amount of the ethyl-methoxide or -ethoxide and they collectively account for over 90% of the radioactivity in the ether-soluble fraction. In each case there are at least eight very minor unidentified metabolites. The ethyl-epoxide yields the ethyl-diol as the major metabolite in *Tenebrio* pupae. Thus, the high epoxide hydratase activity limits the persistence of the ethyl-epoxide while the ethyl-methoxide and -ethoxide are more stable, undergoing primarily *O*-dealkylation presumably by the action of microsomal oxidases.

Enzyme studies. The housefly microsome–NADPH system metabolizes the ethyl-methoxide to a greater extent than the ethyl-ethoxide and with greater specificity for *O*-dealkylation in the former case (Table 2). Minor metabolites in the housefly microsome system arise from benzylic oxidation. Mouse microsomes behave in a similar manner, but there is a higher degree of benzylic oxidation. One of the mouse metabolites of the ethyl-alkoxides, accounting for 4–10% of the methoxide metabolites and 10–18% of the ethoxide metabolites, chromatographs as anticipated for the 2,3-epoxy derivative without additional modifications. Under comparable conditions the ethyl-epoxide products from mouse liver microsome–NADPH metabolism consist of: 37% unmetabolized ethyl-epoxide, 10% ethyl-diepoxide, 20% α-hydroxyethyl-epoxide, 3% aceto-epoxide, 5%

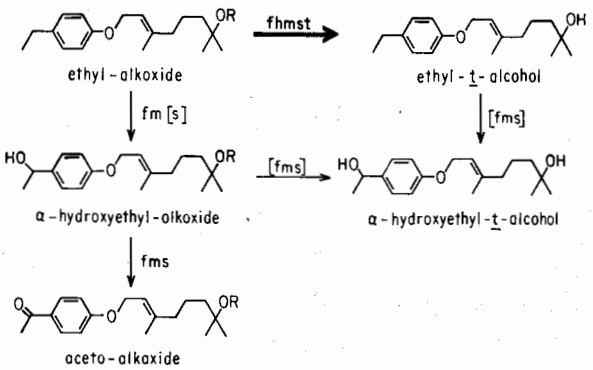


FIG. 4. *Tentative metabolic pathways for the ethyl-methoxide* ($R = CH_3$) *and ethyl-ethoxide* ($R = C_2H_5$). *The systems are:* f *and* m-*housefly and mouse liver microsome-NADPH reaction mixtures*; h, s *and* t, *housefly adult, stable fly adult, and* Tenebrio *pupae* in vivo. *Brackets designate alternative pathways to the same end product.*

ethyl-diol, and 25% α-hydroxyethyl-diol and unidentified metabolites. The metabolism of the ethyl-alkoxides is dependent on the presence of both microsomes and NADPH while that of the ethyl-epoxide requires the addition of NADPH to the microsomes only for reactions other than epoxide hydration.

Determination of the influence of substrate concentration (3–500 μM) on the housefly microsome–NADPH system led to sigmoidal Lineweaver–Burk plots; however, at each substrate level the order of preference as a substrate decreases in the order: diene > epoxide ≫ methoxide > ethoxide.

Photodecomposition. The ethyl-epoxide, -methoxide, and -ethoxide do not decompose appreciably on silica gel when held in the dark for up to 12 hr but they photodecompose when exposed to sunlamp irradiation, with the times for 25% conversion to photoproducts being 7.8, 8.3, and 11.4 hr, respectively. The photoproducts of the ethyl-methoxide and -ethoxide are generally polar compounds and do not include any of the available authentic standards.

DISCUSSION

Metabolism of the ethyl-alkoxides involves *O*-dealkylation as the major pathway but benzylic oxidation before or after *O*-dealkylation is also important, both *in vivo* and *in vitro* (Fig. 4). The ethyl-epoxide is easily degraded under acidic conditions or by the action of epoxide hydratases while the ethyl-alkoxides undergo *O*-dealkylation. Thus, the alkoxides are expected to be rapidly metabolized and possibly more rapidly than the epoxide in insects with high oxidase levels such as polyphagous insects and those previously selected with insecticides.

The ethyl-ethoxide is an extremely potent juvenoid with *T. molitor* but it shows little or no activity with three species of Lepidoptera, three of Diptera, and two of Coleoptera (6, 8, 12, 13); in contrast, the ethyl-epoxide shows moderate to high activity with a variety of insects (3, 4, 14). Rapid metabolism probably does not account for the inactivity of the ethyl-ethoxide in all of the insensitive insects. Thus, it is essentially inactive with housefly and stable fly pupae (12, 13) while persisting considerably longer than the ethyl-epoxide in adults of these species. A portion of the specificity may be due to fit at the hormone receptor since the ethyl-methoxide and especially the ethyl-ethoxide show very low activity in blocking the β-ecdysone-induced evagination of *Drosophila melanogaster* imaginal disks when compared to the ethyl-epoxide and a natural juvenile hormone (6).

The high morphogenetic activity of the ethyl-epoxide compared to other *p*-substituted phenyl epoxygeranyl ethers may be due in part to the compound entering a pool in the insect where it is refractory to degradation (6). The less rigid requirement for a particular *p*-substituent in the phenyl ethoxygeranyl and *n*-propoxygeranyl ether series may be attributable to their very slow degradation in *T. molitor* regardless of whether they enter the refractory pool, so that with this species the rates of degradation have little if any bearing on structure–function relationships among the alkoxides. The slightly higher activity of the ethyl-ethoxide over the ethyl-propoxide and the low activity of the ethyl-isopropoxide and very low activity of the ethyl-methoxide (6, 8) are more attributable to fit at hormone receptor sites than to metabolism.

The ethyl-ethoxide and related alkoxides provide a means for overcoming the lability of the epoxide substituent in the analogous epoxy compounds. While this is advantageous in improving potency and persistence in some cases, it may also reduce the spectrum of sensitive insects by providing less satisfactory fit at the hormone receptor site and a substituent easily detoxified by *O*-dealkylating oxidases.

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