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-menthygeraniol, and 7-\(\alpha\)-hockeygeraniol.

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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 co-chromatographic 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-c-carboxylic acid and metabolites cochromatographing with it were verified by their reaction with OsO\(_4\), to form the ethyl-triol and their failure to be acylated with acetic anhydride-pyridine, while metabolites cochromatographing with the ethyl-triol were confirmed by preparation of their \(\beta\)-butyrolactone ester (8).

Abbreviations used: BPA, \(\beta\)-butyrolactone acid; PE, p-ethoxy-ether-ether; BP, benzene-propenyl;
The [4H][1,2,4]triazolo[3,4-b]pyridin-4-amine (0.05 mol) and ethyl vinyl ether (0.05 mol) were heated in a sealed tube at 160°C for 4 hours. The reaction was monitored by thin-layer chromatography (TLC) using silica gel plates.

To a solution of the reactants in dry THF (20 mL), triethylamine (2.0 mL) was added. The mixture was stirred at room temperature for 30 minutes. The resulting mixture was poured into water and extracted with dichloromethane. The combined extracts were washed with brine, dried over anhydrous 

The [4H][1,2,4]triazolo[3,4-b]pyridin-4-amine was then isolated by column chromatography on silica gel (100-200 mesh). The eluent was a mixture of dichloromethane and methanol (95:5). The pure product was obtained in 85% yield.

The obtained [4H][1,2,4]triazolo[3,4-b]pyridin-4-amine was recrystallized from methanol to give colorless needles (m.p. 200°C). The purity of the product was confirmed by 

The [4H][1,2,4]triazolo[3,4-b]pyridin-4-amine was then subjected to a series of reactions to prepare the desired [4H][1,2,4]triazolo[3,4-b]pyridin-4-amine derivatives. These reactions involved the introduction of various functional groups, such as 

The [4H][1,2,4]triazolo[3,4-b]pyridin-4-amine derivatives were then evaluated for their biological activity. The results showed that these compounds exhibited 

In conclusion, the synthesis of [4H][1,2,4]triazolo[3,4-b]pyridin-4-amine derivatives was successful and provided a new series of compounds with potential 

Further studies are ongoing to optimize the reaction conditions and to investigate the biological activities of these compounds in greater detail.
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 homogenously 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 niotinamide-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 0.1% bovine serum albumin (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 (NH4)2SO4 and ether (10 ml). The ether extract was analyzed by tlc followed by hplc.

Photostability. In accordance with the

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**Figure 1.** Synthesis routes for unlabeled standards, [3H]ethyl-α-lactone and [3H]ethyl-α-thiol. The α-thiol series in which the pc(4-hydrazinobenzyl) is prepared in the same manner from the aminodiol. The α-thiol series is converted to the α-hydroxyethyl pc-C6-H5H4O2- series on reduction with LiAlH4.

**Figure 2.** Separation of ethyl-naphthoate, ethyl-ethanoate and several of their metabolites and derivatives on silica gel F-254 chromatoplates.
procedure of Gill et al. (5), a spot (40 n mole) of the ᵃH 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 3 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-epoxide 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 ᵃH 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 = CH₂CH₃, R = CH₃) and Ethyl-Alkoxides (X = R = CH₂CH₃) in Living Stable Flies and in Microsome-NA DPH Systems of Houseflies and Mouse Liver

<table>
<thead>
<tr>
<th>X</th>
<th>R</th>
<th>Livings stable Sy.</th>
<th>Microsome-NA DPH system</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2 hr</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>CH₃ CH₂CH₃</td>
<td></td>
</tr>
<tr>
<td>CH₃CH₂</td>
<td>CH₃ or CH₃</td>
<td>CH₃, CH₂CH₃</td>
<td>CH₃, CH₂CH₃, CH₃CH₃</td>
</tr>
<tr>
<td>CH₂CH₃</td>
<td>H</td>
<td>27</td>
<td>23, 7</td>
</tr>
<tr>
<td>CH₂CH₃</td>
<td>CH₃ CH₃</td>
<td>&lt;1</td>
<td>1, 2</td>
</tr>
<tr>
<td>CH₃CH₂</td>
<td>CH₃ CH₃</td>
<td>&lt;1</td>
<td>0.2</td>
</tr>
<tr>
<td>CH₃CH₃</td>
<td>R</td>
<td>0.5</td>
<td>1, 2</td>
</tr>
<tr>
<td>CH₃CH₃</td>
<td>CH₃ CH₃</td>
<td>&lt;1</td>
<td>4</td>
</tr>
<tr>
<td>Unidentified</td>
<td></td>
<td>7</td>
<td>4</td>
</tr>
</tbody>
</table>

Organosoluble radioactivity recovered (%), with indicated R-substituent.
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% acetox-epoxide, 1.3% ethyl-disepoxide, 0.4% α-hydroxyethyl-diol, and 13.5% as 23 unidentified ether-soluble products.

In mealworms at 36 hr after treatment the ethyl-alcohol from O-dealkylation is present in about one-half the amount of the ethyl-nmethoxide or -ethoxide and they collectively account for over 90% of the radioactivity in the ether-soluble fraction. In each case there is 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 hydrolase activity limits the persistence of the ethyl-epoxide while the ethyl-nmethoxide and -ethoxide are more stable, undergoing primarily O-dealkylation presumably by the action of microsomal oxidases.

Enzyme studies. The housefly microsomal-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 benzyl oxidation. Mouse microsomes behave in a similar manner, but there is a higher degree of benzyl oxidation. One of the mouse metabolites of the ethyl-alkoxide, accounting for 4-10% of the methoxide metabolites and 10-18% of the ethoxide metabolites, chromatographs as anticipated for the 2,3-epoxy derivative with additional modifications. Under comparable conditions the ethyl-epoxide products from mouse liver microsome-NADPH metabolism consist of: 37% unmetabolized ethyl-epoxide, 10% ethyl-disepoxide, 20% α-hydroxyethyl-epoxide, 3% acetox-epoxide, 5%
ethanol-diol and 25% 5-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 (0-500 μM) on the housefly microsomal-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 > methionine > ethoxide.

Pharmacokinetics. 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 sunlight in air, 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 vitro and in vivo (Fig. 4). The ethyl-epoxide is easily degraded under acidic conditions or by the action of epoxide hydrolases 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 insesticides.

The ethyl-ethoxide isan extremely potent juvenile with 7, 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-ethoxide and especially the ethyl-ethoxide shows 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 aldehydes. The slightly higher activity of the ethyl-ethoxide over the ethyl-propoxizide and the low activity of the ethyl-isopropoxizide and very low activity of the ethyl-naphoxizide (6, 8) are more attributable to fit at hormone receptor sites than to metabolism. The ethyl-ethoxide and related aldehydes provide a means for overcoming the liability 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.

REFERENCES
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