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PRELIMINARY CHROMATOGRAPHIC STUDIES ON THE METABOLITES AND PHOTODECOMPOSITION PRODUCTS OF THE JUVENOID 1-(4'-ETHYLPHENOXY)-6,7-EPOXY-3,7-DIMETHYL-2-OCTENE

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Abstract

Several metabolites and photodecomposition products of the juvenoid, 1-(4'-ethylphenoxy)-6,7-epoxy-3,7-dimethyl-2-octene, labeled with ^{14}C or ^{3}H in the phenyl group or with ^{3}H in the 1-position of the octene moiety, were tentatively identified by thin-layer chromatographic comparison with 12 authentic, unlabeled compounds from synthesis. On incubation with the rat liver microsome or soluble fraction, the juvenoid is converted to two metabolites, one of which is the diol formed by hydration of the epoxide moiety. Addition of reduced nicotinamide-adenine dinucleotide phosphate to the microsome incubation mixture results in formation of: the diol; 1-(4'-ethylphenoxy)-2,3,6,7-tetrahydroxy-3,7-dimethyloctane; 1-[4'-(1-hydroxyethyl)-phenoxy]- and 1-(4'-acetophenoxy)-6,7-epoxy-3,7-dimethyl-2-octenes; at least 9 other metabolites retaining the ether linkage (detected with both ^{3}H-phenyl and ^{3}H-octene preparations). Rats administered the ^{3}H- or ^{14}C-juvenoid intraperitoneally excrete almost all of the radioactivity within 54-96 hr, about equally in urine and feces. The 6 or more ^{3}H-phenyl-labeled metabolites in urine are not identified but they are relatively polar compounds. The diol is one of many metabolites excreted in rat and locust feces. Photodecomposition of the juvenoid as a deposit on silica gel chromatoplates occurs slowly in sunlight unless a photosensitizer (xanthone, anthraquinone) is added in

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which case it occurs rapidly forming 1-[4'-(1-hydroxyethyl)-phenoxy]- and 1-(4'-acetophenoxy)-6,7-epoxy-3,7-dimethyl-2-octenes and at least 6 other products retaining the ether linkage. The sites in this juvenoid most susceptible to degradation appear to be the epoxide moiety, the benzylic methylene group, and possibly the trans-olefin group.

Introduction

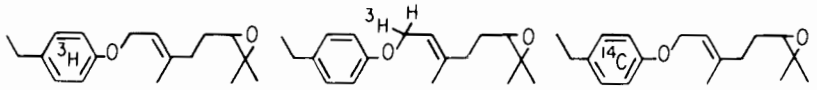
A wide variety of juvenile hormone analogs (juvenoids) have morphogenetic activity on certain insects under laboratory conditions. Juvenoids may be useful as ovicides, mosquito larvicides, in household pest control, and as protectants for livestock, stored grains, and agricultural crops. However, the effectiveness of juvenoids for control of agricultural insect pests under field conditions has been somewhat disappointing. This may be because the juvenoid, once applied to crops, fails to persist for the requisite time to contact most individuals of the population at sensitive stages of development. Since the use of juvenoids in insect control will potentially result in residues on or in food, feed and forage, it is important to understand the mechanisms and pathways for their metabolism and photodecomposition. Information of this type is being developed by several laboratories (1,2).

The juvenoid considered in this report is 1-(4'-ethylphenoxy)-6,7-epoxy-3,7-dimethyl-2-octene (Compound I of Fig. 1; R-20458 of Stauffer Chemical Co.; referred to in this communication as the juvenoid), a relatively simple but yet very potent morphogenetic agent (3). The degradation chemistry studies involved incubation of the juvenoid with rat liver enzymes, administration to rats and locusts to obtain excreted metabolites, and exposure to sunlight.

Materials and Methods

Chemicals. The structures for the chemicals used are given in Fig. 1. Although some of the compounds were cis- trans-mixtures, the trans isomer was predominant in each case.

Three radiolabeled preparations (A, B and C) of compound I were used.



A B C

The ^{3}H-phenyl preparation (A, 654 mCi/mmole) was prepared from ring-labeled ^{3}H-ethylphenol, obtained by an exchange reaction under acidic conditions, and the ^{3}H-geranyl preparation (B, 33 mCi/mmole) was made from ^{3}H-geraniol containing some ^{3}H-nerol obtained by reducing citral with $NaB^{3}H_4$ (4). Stauffer Chemical Co. (Mountain View, Calif.) provided the ^{14}C-labeled preparation (C, 17 mCi/mmole).

Several unlabeled derivatives or possible degradation products of compound I were prepared for use in co-chromatographic comparisons with radiolabeled metabolites and photodecomposition products. The corresponding diene (II) (3,4) was converted to the diepoxide derivative (III) by oxidizing it with 3 molar equivalents of m-chloro-peroxybenzoic acid in chloroform solution. Three methods were used to prepare diol IV: reacting the corresponding diene (II) with equimolar osmium tetroxide in the presence of 2 molar pyridine in benzene solution and transesterification of the osmate ester with mannitol; hydrolysis of the epoxide (I) in 0.5N H_2SO_4 in 40% aqueous tetrahydrofuran at 25°C for 10 min; hydrolysis of the epoxide (I) in 10% aqueous ethylene glycol containing 0.5% KOH at 120°C for 3 hr. The tetraol (V) was made from the diepoxide (III) by hydrolysis in 0.5N H_2SO_4 in 40% aqueous tetrahydrofuran at 25°C for 10 min. Reduction of the epoxide (I) with $NaBH_4$ in ethanol yielded the tertiary alcohol (VI). Titration of the diol (IV) with lead tetraacetate in benzene converted it quantitatively to the aldehyde (VII) which was oxidized with excess silver nitrate and ammonium hydroxide (Tollens' reagent) to form the acid (VIII). The diene resulting from reaction of 4-hydroxyacetophenone with geranyl bromide was either epoxidized to obtain compound IX (5) or reduced with $NaBH_4$ and then epoxidized to obtain compound X. Reaction of geranyl bromide with the methyl ester of 4-hydroxyphenylacetic acid, hydrolysis of the ester

group and epoxidation gave compound XI. Compound XII was made by reacting potassium 4-ethylphenylate with the methyl ester of bromoacetic acid and subsequent hydrolysis of the methoxycarbonyl group. The compounds were isolated in pure form by preparative thin-layer chromatography (TLC) on silica gel GF chromatoplates (2 mm gel thickness, Analtech, Inc., Newark, Del.) or by column chromatography on dry-packed Florisil (60/100 mesh, Floridin Co., Berkeley Springs, W. Va.) columns developed with hexane-ether or ether-methanol gradients. In each case, infrared, nuclear magnetic resonance and mass spectra confirmed the identity of the synthesized products.

Analytical methods. Metabolites and photodecomposition products were separated by two-dimensional TLC on silica gel F_{254} chromatoplates (0.25 mm gel thickness, E. Merck, Darmstadt, Germany) developing with the following sequence of solvent systems: benzene-n-propanol (20:1) (referred to as BP) in the first direction; chloroform-dichloromethane-ethyl acetate-n-propanol (10:10:1:1) (CDAP) in the second direction; carbon tetrachloride-ether (2:1) (TE) as a second development in the first direction. Radioactive compounds were detected by radioautography (6) and authentic unlabeled compounds were detected with appropriate chromogenic reagents (7) including: molybdophosphoric acid and heat yielding blue spots on a yellow background; anisaldehyde, sulfuric acid, acetic acid and heat, yielding spots of various colors with different compounds, i.e. green for I and X, blue for IX, violet for II, yellow for III, IV and V, brown for VI and VII, and grey for XI; lead tetraacetate in benzene giving white spots on a brown background with much higher sensitivity for the 6,7-dihydroxy compounds (IV and V) than for the other materials. The labeled metabolites and photodecomposition products derived from ^{3}H-compound A were subjected to cochromatographic comparisons with authentic unlabeled compounds from synthesis for tentative identification of the labeled compounds.

Enzyme studies. Livers of male albino rats were homogenized at 20% (w/v) in potassium phosphate buffer (0.1M, pH 7.4) and the homogenate was centrifuged at 15,000 g for 20 min. The supernatant was decanted

off and centrifuged at 105,000 g for 1 hr to separate the microsomal and soluble fractions. The microsomal fraction was resuspended in phosphate buffer while the soluble fraction was further purified before use by passing it through a Sephadex G-25 column.

Each incubation flask contained the following constituents in a 2.0-ml total volume of phosphate buffer: the microsome and/or the soluble fraction (approximately 10 mg total protein), $MgCl_2$ (21 μmoles), reduced nicotinamide-adenine dinucleotide phosphate (NADPH, 0 or 0.5 μmole), and the substrate (labeled compound A, B or C mixed with some unlabeled material to give a total of 0.1 μmole; added in 10-25 μl ethanol). After incubation at 37°C for 2 hr, the reaction mixtures were frozen and lyophilized, followed by extraction of the residue with methanol. The methanol extract was concentrated under nitrogen and analyzed for radioactive components by TLC.

In vivo studies with rats and locusts. Male albino rats (250 g) were treated intraperitoneally with the labeled juvenoid (A, B or C) at 10-500 mg/kg administered as a solution in corn oil. Urine and feces were collected at 24 hr intervals for up to 96 hr. The level of radioactivity in the urine and feces was determined relative to that in the administered dose. In another study, the rats were treated with ^3H-compound A at 1 mg/kg, using ethanol as the administration vehicle, and the urine and feces were collected for 96 hr after treatment. The urine was lyophilized and the residue extracted with methanol. The feces were dried at reduced pressure over phosphorus pentoxide and extracted with methanol. The methanol extracts were analyzed by TLC.

Adult male desert locusts (Schistocerca americana) were injected with the ^3H-labeled juvenoid (A, 1 μg in 1 μl ethanol), the feces collected for 48 hr after treatment were subjected to continuous Soxhlet extraction with hexane, and the metabolites were then extracted from the hexane into acetonitrile for analysis by TLC.

Photodecomposition. The labeled compound (A,B or C) was applied at the origin of a silica gel F_{254} chromato-

plate to yield a residual deposit of 0.12 μmole/cm^2, in the presence or in the absence of a potential photosensitizing chemical (xanthone or anthraquinone, 0.06 μmole/cm^2). Following exposure in the dark or to direct sunlight for 8 hr, the chromatogram was developed to separate the products.

Results

Chromatographic separation of some of the possible metabolites and degradation products. The chromatographic positions of the various authentic compounds are shown in Fig. 1. The solvent systems are not appropriate for polar compounds such as V, XI and XII. Also, they fail to separate the aldehyde derivative (VII) from the parent compound (I).

Metabolites formed by rat liver enzymes. The epoxide (I) is converted, in small amount, by the microsomes without NADPH to two metabolites, one of which cochromatographs with the diol (IV) and the other, which is unidentified, chromatographs slightly above the diol. The soluble fraction also yields the diol (IV) and another metabolite which may be the same as the unidentified product formed by the microsomes alone. Addition of NADPH to the microsomal system results in the formation of at least 14 metabolites present in greater than 1% yield (Fig. 2). The same two metabolites are detected in the microsome- or soluble enzyme system and the same 14 metabolites are detected in the microsome-NADPH system using any one of the ^{3}H-phenyl- (A), ^{3}H-geranyl- (B), or ^{14}C-phenyl (C) preparations; so, each metabolite is an ether. The product of higher Rf than the parent compound (I) is not evident in all studies and so it is not known whether it is a metabolite or originates from other sources. The total radioactivity accounted for in the products separated by TLC was 62% of the amount used in the incubation mixture.

The metabolite of the juvenoid (I) formed most easily by the microsome system without NADPH and by the soluble fraction was found to cochromatograph with diol IV, as indicated above. When this metabolite derived from the ^{14}C-substrate (C) was isolated, by TLC, it was found to

cochromatograph with the diol (IV) on direct spotting, with the corresponding aldehyde (VII) after oxidation with lead tetraacetate, and with the corresponding acid (VIII) after subsequent oxidation with Tollens' reagent. These derivatives confirm the identity of the diol.

Other metabolites formed in the microsome-NADPH system cochromatograph with the 1-[4'-(1-hydroxyethyl)-phenoxy]-

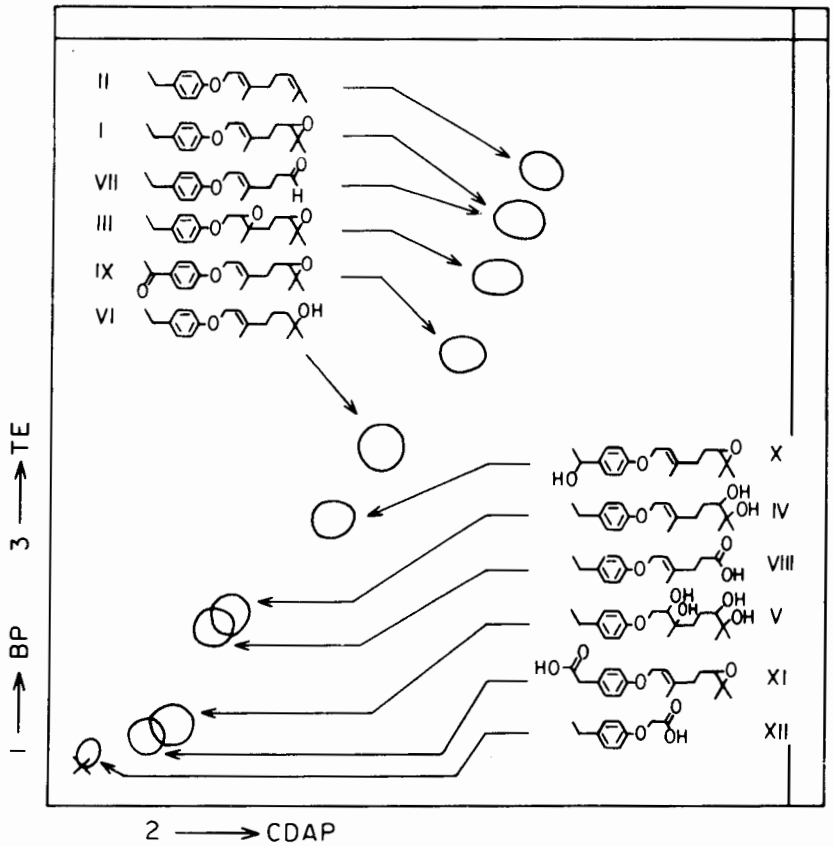


Fig. 1. Chromatographic positions and structures for 1-(4'-ethylphenoxy)-6,7-epoxy-3,7-dimethyl-2-octene (I) and related compounds (II-XII).

and 1-(4'-acetophenoxy)-6,7-epoxy-3,7-dimethyl-2-octenes (X and IX, respectively) and possibly with the tetraol (V).

Metabolites excreted by rats and locusts following injection of the juvenoid. Table 1 gives the data from

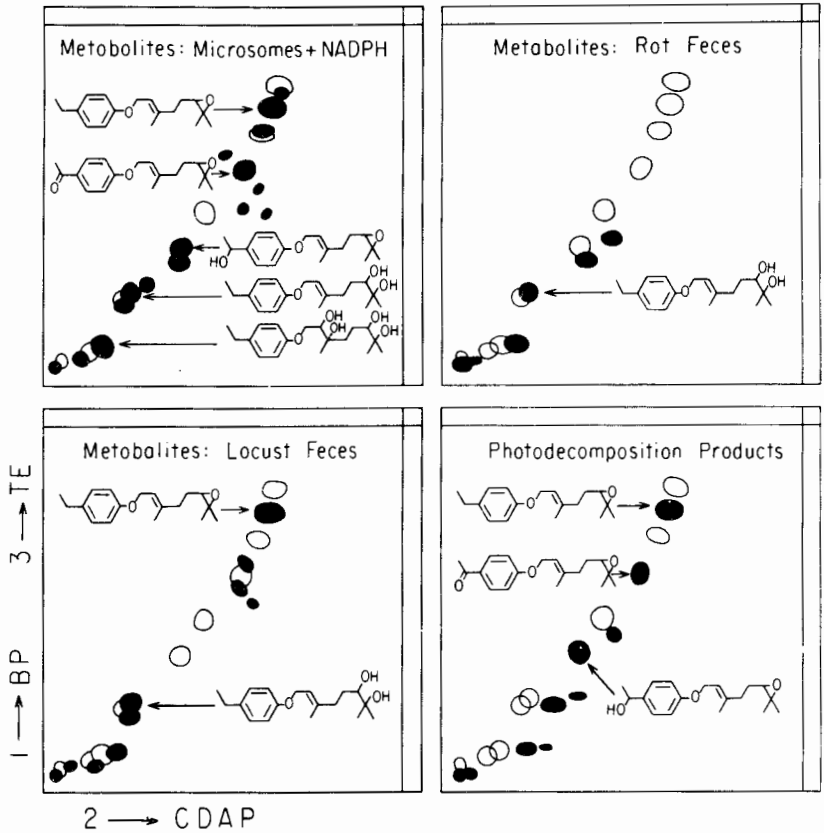


Fig. 2. Chromatographic positions for metabolites and photodecomposition products of 1-(4'-ethylphen-^{3}H-oxy)-6,7-epoxy-3,7-dimethyl-2-octene (darkened circles) and the structures for compounds cochromatographing with individual components. The positions for known compounds not cochromatographing with metabolites and photodecomposition products are shown as open circles (see Fig. 1).

studies in which varying doses of labeled compounds A, B and C were administered intraperitoneally to rats and the level of radioactivity in the urine and feces was determined for up to 96 hr thereafter. The radioactivity of the ^{14}C- and ^{3}H-phenyl labeled compounds (C and A) is largely, if not completely, eliminated from the body within 54-96 hr, but a smaller amount of the radioactivity is accounted for in the excreta of rats treated with the ^{3}H-geranyl labeled preparation (B). The urine contains many metabolites, but none of these chromatograph with Rf values higher than that of the tetraol (V) under the standard conditions. Using repeated development with benzene-n-propanol (10:1) mixture in the same direction, the polar urinary metabolites are resolved into at least 6 components, no one of which is identified. The extracts of rat and locust feces contain many metabolites (Fig. 2) including one cochromatographing with the diol (IV) in each case and others chromatographing above and below this position.

There are preliminary indications of a specific tissue

TABLE 1

Balance study on excretion of radioactive compounds in the urine and feces of rats dosed intraperitoneally with ^{14}C- or ^{3}H-labeled 1-(4'—ethylphenoxy)-6,7-epoxy-3,7-dimethyl-2-octene[a]

Labeling position	Excreted radioactivity, %		
	Urine	Feces	Total
^{14}C-Phenyl (C)[b]	50	50	100
^{3}H-Phenyl (A)[c]	62	34	96
^{3}H-Geranyl (B)[c]	40	33	73

[a]Studies conducted by L. J. Hoffman and J. J. Menn.
[b]The dose was 46 mg/kg and the excreta were collected for 96 hr.
[c]The excretion results are averaged for administered doses of 10, 50, 250 and 500 mg/kg. The excreta were collected for 54 hr.

localization of the ^{3}H from labeled compound A in locusts injected 3 weeks after allatectomy as newly-molted adults. This localization, when it occurs, appears to be specific for the testes and persists for several days; no comparable persisting ^{3}H levels appear in other tissues of the males or in any tissues of the females. The chemical basis for and significance of this localization are unknown.

Photodecomposition. The juvenoid does not photodecompose appreciably when exposed to sunlight for up to 8 hr (3) on a silica gel chromatoplate in the absence of a photosensitizer, or in the dark in the presence of a photosensitizer, but extensive degradation results when xanthone or anthraquinone is added to the juvenoid and it is then exposed to sunlight. At least 8 photodecomposition products are formed in the presence of xanthone (Fig. 2) and two of these cochromatograph with the 1-[4'-(1-hydroxyethyl)-phenoxy]- and 1-(4'-acetophenoxy)-6,7-epoxy-3,7-dimethyl-2-octenes (X and IX, respectively). The remaining photodecomposition products are not identified but they do not include the diepoxide (III) or any of the other authentic compounds available. All of the major photodecomposition products are ethers because they are detected with both the ^{3}H-phenyl (A) and ^{3}H-geranyl (B) preparations. There are also other photodecomposition products appearing after shorter irradiation periods but these appear to be transient materials leading to the formation of more stable derivatives.

Discussion

The emphasis in the present study was placed on relatively apolar metabolites and photodecomposition products retaining the ether linkage. Thus, relatively polar products such as glucuronide, glucoside, sulfate and other conjugated metabolites and polar polymeric photodecomposition products are not considered. The cochromatographic technique employed is not adequate, by itself, for identification of the metabolites and photodecomposition products. However, it does indicate that the sites on the juvenoid most susceptible to attack, depending on the conditions, include the epoxide moiety,

the benzylic methylene group, and possibly the trans-olefin group.

The ether group is not cleaved to an appreciable degree in the enzymatic systems studied; so, there is not extensive oxidation at C-1 of the geranyl-derived moiety. Epoxide cleavage by the liver soluble and microsomal fractions possibly results from the action of a "hydrase"; similar cleavage by insect enzyme preparations forms the corresponding diol from Cecropia juvenile hormone (2). The enzymatic conversion of the 4-ethylphenoxy moiety to the 4-acetophenoxy moiety via the corresponding alcohol is anticipated from studies on other 4-ethylphenoxy compounds (8). The reactions occurring in living rats and locusts seem to be similar, in part, to those encountered with the microsome-NADPH system. It is obvious from the number of metabolites that other sites on the molecule are also susceptible to attack or that some intramolecular rearrangements take place during the course of metabolism. However, many of the metabolites could be formed by combinations of attack at the sites already defined.

A specific inhibitor capable of blocking the enzymatic metabolism of this type of juvenoid would be potentially useful in prolonging the duration of juvenoid action in insects. It appears unlikely that a single compound would be adequate to block all of the enzymes involved in juvenoid breakdown by the liver system investigated. However, an inhibitor for the most rapid reaction in a particular enzyme system (or species in vivo) might extend the persistence of the juvenoid and the epoxide cleavage reaction is a candidate for this purpose. The imino analog of the epoxide is of potential interest in this respect (9).

The methylene position of the ethylphenoxy moiety undergoes sensitized photochemical oxidation and this occurs, in part, without other modification of the molecule. The diepoxide is not detected among the photodecomposition products of compound I (this report) but it is with a related compound (1).

The activities of several of the possible metabolites

and degradation products of juvenoid I in producing 50% pupal-adult intermediates when applied topically to Tenebrio molitor pupae are as follows, as μg/pupa: I-0.007; II and IX - 0.3-0.5; III - 10; VII - 80; IV, V, VIII and XII - > 100. The activities found for compounds I, II, and IX are in general agreement with earlier reports (3, 5). Within the limitations of this test, the products are all of reduced biological activity. It is of interest to test these compounds for potency on phytoplankton because compound I at 10 ppm inhibits the growth of Chlamydomonas sp.

The juvenoid investigated (compound I) represents a class of many related compounds with high morphogenetic activity (3,5). The reactions noted with this compound should therefore be applicable, in part, to other 1-(substituted-phenoxy)-6,7-epoxy-3,7-dialkyl-2-octenes where the alkyl substituents are methyl or ethyl.

Acknowledgement

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References

1. B. A. Pawson, F. Scheidl, and F. Vane, this volume.
2. M. Slade and C. H. Zibitt, this volume.
3. F. M. Pallos, J. J. Menn, P. E. Letchworth and J. B. Miaullis, Nature 232, 486 (1971).
4. H. Kamimura, B. D. Hammock, I. Yamamoto and J. E. Casida, J. Agr. Food Chem., in press.
5. W. S. Bowers, Science 164, 323 (1969).
6. M. Elliott and J. E. Casida, J. Agr. Food Chem., in press.
7. E. Stahl, "Thin-Layer Chromatography", Springer-Verlag, New York, 1969.

8. M. Eto and M. Abe, Biochem. Pharmacol. 20, 967 (1971).
9. E. J. Corey, First Swiss Symposium on Juvenile Hormones, Basle, October 17, 1970.