THE DEGRADATIVE METABOLISM OF JUVELINOIDS BY INSECTS

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Summary

In general, insects metabolize juvenoids in the same manner as other xenobiotics. Ester cleavage, epoxide hydration, olefinic isomerization, and various oxidative pathways have been established for juvenoid metabolism with metabolic pathways largely determined by the functionality available for biochemical modification in each juvenoid. It cannot be generalized that a given metabolic pathway is most important for all juvenoid degradation, since biologically active juvenoids exist in the absence of specific functional groups (e.g., R-20458 lacks ester and methoprene lacks epoxide). Thus, the basis for JH activity in each juvenoid must be considered separately and the importance of various metabolic routes can only be measured empirically until more is known about the molecular structure-activity relationships for natural JH's.
gas-liquid chromatography (gbc) has been used infrequently in JH or juvenoid work in spite of its resolving power (Slade and Zibitt, 1972; White, 1972; Kamimura et al., 1972). Terriere and Yu (1973) found that gbc with an electron capture detector is quite sensitive for detecting juvenoids with conjugated systems as methyrene, but unfortunately this procedure is not useful for examining metabolites of variable polarities or metabolites from systems with contaminating materials. Hoffman et al. (1973) made extensive use of electron impact mass spectroscopy (ms) for metabolite identification, and chemical ionization ms is very useful for some polyhydroxylated juvenoid metabolites (Hammock unpublished). One can predict more extensive use of ms and especially gbc/ms as such instruments become more sensitive and accessible.

Initiation of the investigation of juvenoid metabolism coincided with the first intensive use of high resolution liquid chromatography (hrlc), and the Zoecon group has done a great deal to apply this powerful tool to the JH's and juvenoids (Dunham et al., 1975). Gel permeation, adsorption, and reversed-phase hrlc are useful in the purification of juvenoid metabolites and their tentative identification by co-chromatography. The detection limit at the present time for UV absorbing juvenoids is in the nanogram range which is useful for monitoring some relatively clean enzyme systems as epoxide hydratases (Hammock, unpublished), but is generally not sensitive enough for most metabolism work.

The procedures involved in the elucidation of pathways involved in the metabolism of JH's and juvenoids are common to most studies of xenobiotic metabolism with a heavy emphasis on radiotracers. The recurrent problem through most JH and juvenoid studies is that the specific activities of the radiolabels used are in many cases not high enough to permit study at low dose levels consistent with the physiological action of these compounds.
METABOLISM OF FUNCTIONAL GROUPS OF JUVENOIDS

Metabolism of the Ester Functionality

A major route of juvenile hormone (JH) degradation in all insects examined except possibly the higher Diptera is ester cleavage (Slade and Zibitt, 1971, 1972; White, 1972; Ajami and Riddiford, 1973). As an example, JH metabolism in Manduca sexta rapidly converts JH I in vivo to its JH acid (2, Fig. 1) and JH acid diol (3, Fig. 1). The haemolymph of M. sexta contains high levels of soluble esterases capable of hydrolyzing JH (Weirich et al., 1973; Sandburg et al., 1975) while the fatbody contains both esterases and epoxide hydratase. The 12,000 g pellet ("mitochondria"), 100,000 g pellet ("microsomes") and 100,000 g supernatant (soluble) fractions of M. sexta fatbody are all capable of hydrolyzing JH to JH acid (Hammock et al., 1975b) indicating the presence of both membrane-bound and soluble esterases.

The first report of a correlation between activity and metabolic stability of juvenoids was made by Weirich and Wren (1973). They reported that the esterases in M. sexta haemolymph rapidly hydrolyzed JH I and that the ethyl ester of JH I (3, Fig. 2) appeared to be hydrolyzed more slowly than the methyl ester while the isopropyl ester of the juvenoid methoprene (1, Fig. 2) was not hydrolyzed. This observation points to a general trend in esterases that hydrolysis rate is often methyl > ethyl > propyl >> isopropyl although hydrolysis of the isopropyl ester, methoprene, cannot be compared directly with the methyl and ethyl esters of JH. Weirich and Wren also found that the natural 2E isomer of JH I was very rapidly hydrolyzed while the 2Z isomer was not. This finding has been confirmed in several other Lepidoptera for methyl farnesoate and indicates a rather marked substrate specificity for JH esterases.

The ester functionalities of JH's are distinct in that they are conjugated with the 2,3-double bond providing more chemical stability than corresponding unconjugated esters, and this conjugation may explain the marked stability of
JH to many general esterases as reported for *M. sexta* haemolymph by Sandburg et al. (1975). The stability is also exemplified by the lack of degradation of JH by mammalian hepatic soluble esterases (Hammock et al., 1974c), *Drosophila pseudobscura* locus E5 esterases (Marshman, unpublished observation), and partially purified esterases from *Musca domestica*, *Blaberus giganteus*, and *Meliitthia rea* which rapidly hydrolyze α-naphthol acetate and g-nitrophenyl acetate (Munby and Hammock, unpublished observations). With regard to *D. pseudobscura* and *M. domestica* it should be remembered that Slade and Zibitt (1972) found that ester cleavage is a very minor route of JH I metabolism in another Dipteran *Sarcophaga bullata*.

In most insects the hydrolysis of the methyl ester is a major route of JH metabolism, especially in those stages when the insect appears most sensitive to JH or juvenoid treatment. Therefore, other alcohols have been used in synthetic juvenoids partially to circumvent the facile hydrolysis of methyl esters. Several compounds such as hydropropene and Ro-8-4314 (4, 5 respectively Fig. 2) are ethyl esters, while reminiscent of estrogen work, other compounds (6, 7Fig. 2) are allyl esters. One of the most active juvenoids of many insects is the isopropyl ester methoprene (1, Fig. 2). Unsaturated bonds at the 2 and 4 positions in methoprene and several other juvenoids, result in a conjugated dienoate system which is even more stable to chemical hydrolysis than the conjugated enoate system of the natural JH’s. By monitoring the disappearance of methoprene by electron capture gc from housefly enzyme systems without NADPH, Yu and Terriere (1975) have tentatively indicated the importance of esterases in the metabolism of ethyl, propynyl and thioesters (6, 8, 9, 10, and 2, Fig. 2). The isopropyl ester methoprene is not only refractory to degradation by *M. sexta* haemolymph esterases (Welrich and Wren, 1973) but also esterases from other *M. sexta* tissues (Novock et al., 1975). Solomon and Metcalf (1974) found that a compound co-chromatographing with the methoxy acid was the major in vivo metabolite of methoprene in the milkweed but *G. celtus.*
fasciatus and a minor metabolite in the yellow meal worm Tenebrio molitor. These authors also showed that treatment with triorthocresylphosphate (TOCP), which is a known inhibitor of many insect esterases after metabolic activation, decreased the proportion of acidic metabolites of methoprene in the test insects. Quistad et al. (1975a) found that in Aedes aegypti and Culex pipiens quinquefasciatus larvae the methoprene ester is cleaved to give both the methoxy and the hydroxy acids (2,3 respectively, Fig. 3). Additional evidence for the importance of metabolism of methoprene by esterases in mosquitoes is provided by the slight increase in larval mortality in methoprene-treated larvae when co-treated with TOCP (Quistad et al., 1975a).

Quistad et al. (1975a) found that the major nonpolar metabolite of methoprene from M. domestica post-feeding third-instar larvae is the hydroxy acid. Hammock et al., (1975c) have found that the hydroxy-ester is the major early in vivo metabolite of methoprene in post-feeding larvae of M. domestica, but that more polar products become more important with time. The in vitro systems fail to predict completely the in vivo products as Yu and Terriere (1975) and Hammock et al. (1975c) have failed to find significant ester cleavage of methoprene in homogenates or subcellular fractions of larvae or adult M. domestica. Gel filtration of the soluble fraction from larval M. domestica separated two major esterase fractions, neither showing significant metabolism of methoprene (Hammock et al., 1975c). It is possible that oxidative ester cleavage or methoprene may occur in M. domestica, but this has not been demonstrated in vitro. Perhaps only a brief burst of esterase activity capable of degrading JH or methoprene exists in M. domestica.

Inhibitors of metabolism are commonly used in toxicology as synergists and to indicate the importance of metabolic pathways affecting biologically active compounds. Interpretation of such data
requires cognizance of the possible multiplicity of action of such inhibitors. The probable high cost of manufacturing juvenoids also makes the development of an effective low cost synergist attractive. A number of workers, most recently Ajami (1975), have suggested esterase inhibitors as possible synergists, and the specificity of JH esterases suggests that highly specific JH esterase inhibitors can be found.

Of the juvenoids with an ester functionality so far investigated the isopropyl dienolate structure of methoprene appears to be the most stable; however, many active juvenoids no longer contain the ester functional group. Of these juvenoids, extensive metabolism work has only been reported for the geranyl phenyl ester series.

Metabolism of the Ethylphenyl Moiety

The discovery by Bowers et al. (1969) of the JH activity of the geranyl phenyl ethers has led to the synthesis of a number of very active juvenoids. Although cursory studies were made on unlabeled methylenedioxyphenyl and labeled p-nitrophenyl compounds, (Singh and Hammock, unpublished information, 10, 11, Fig. 2) essentially all of the insect metabolism work with this series has been with the p-ethylphenyl compounds (Hammock et al. 1974a, 1975a). Based on earlier work (Eto and Abe, 1971), it was not difficult to predict the major routes of metabolism of the p-ethylphenyl moiety in insects. N. domestica, Stomoxys calcitrans (stable fly), and Schistocerca americana (American locust) hydroxylate the p-ethylphenyl moiety in the α-position in vivo. The metabolism of the p-ethylphenyl juvenoid R-20658 (2, Fig. 2) has been most extensively studied in the housefly microsomal enzyme system (Fig. 4). Alpha-hydroxylation of the ethyl group is the predominate oxidative pathway while β-hydroxylation is less significant. β-hydroxylation is more pronounced in housefly microsomes than mammalian liver microsomes. Further oxidation of the β-hydroxyethyl compounds to phenylacetic
acids is minor if it occurs at all in housefly microsomes, although this is a pathway in mammals. Addition of NAD and the soluble fraction to the microsomes increases dehydrogenation of \( \alpha \)-hydroxyethylphenyl to acetophenyl derivatives, although this pathway is not as important as with mammalian liver enzymes. S. bullata abdomen microsomes produce the acetophenyl derivative as their major metabolite of R-2045B. No cleavage of the geranyl phenyl ether was detected in housefly microsomes and such cleavage appears to be minor in most insects with the possible exception of P. americana, while in mammals in vivo ether cleavage and \( \alpha \)-oxidation are major degradative pathways (Gill et al., 1972; Hoffmann et al., 1973; Gill et al., 1974; Irive et al., 1975). Ring hydroxylations have not been described for aromatic juvenoids in any insects or mammals examined.

The \( \alpha \)-ethylphenyl moiety of some juvenoids is rapidly metabolized in every mammalian species examined, while its stability varies greatly in insects. It is quite refractory to degradation in T. molitor, but it is very susceptible to \( \alpha \)-hydroxylation and other metabolic pathways in M. domestica.

**Metabolism of the Terminal Functionalities**

The epoxide or oxirane moiety of the juvenile hormone is chemically labile, but it was found quite early to be necessary for high activity in many insects (Bowers et al., 1965). Although several functional groups have been used to replace the epoxide moiety, only the alkoxydes have progressed very far in development (Staal, 1975).

**Metabolism of Epoxides**

Some of the major work on juvenoid and JH epoxide metabolism in insects has been to establish the absence of certain pathways. Reductive opening of epoxides to tertiary alcohols, rearrangement to allylic alcohols, or cyclization analogous to conversion of squalene 2,3-oxide to lanosterol have not been reported
pH optimum 7.9 for JH hydration in *P. eridania* suggesting that different epoxide hydrolases are responsible for JH and HEM metabolism although there certainly may be overlap in activity. Hammock et al. (1974a) found distinct peaks of epoxide hydrolase activity in housefly microsomes at pH's 6.8, 8.1 and 8.9 using R-20458 and its diepoxide as substrates suggesting involvement of multiple enzymes. As with mammalian microsomal hydrolases, insect hydrolases are notable in that they are active at relatively high pH's.

Lack of significant inhibition of R-20458 hydration by 1,1,1-trichloropropane oxide and cyclohexene oxide suggests that the housefly microsomal epoxide hydrolase system is quite distinct from the mammalian epoxide hydrolases responsible for arene oxide metabolism (Hammock et al., 1974a). Slade et al. (1975) also presented evidence for multiple epoxide hydrolases in insects since 1,1,1-trichloropropane oxide inhibits HEM hydration without stabilizing JH in several insect systems.

The metabolism of a juvenoid by epoxide hydration in insects was first demonstrated when the diol of R-20458 (3, Fig. 4) was detected in the feces of *S. americana* (Gill et al., 1972). Brooks (1973) reported circumstantial evidence for the degradation of a juvenoid (7 ethyl homolog of 3, Fig. 2) in homogenates of *Calliphora erythrocephala* and *T. molitor* to a diol while the related radio-labeled compound (3, Fig. 2) is metabolized by epoxide hydration (Hammock, Gill, and Casida, unpublished results) in housefly microsomes. Epoxide hydration of R-20458 has been shown in vitro in nymph and adult *P. americana* and *S. americana* and in larvae, pupae, and adults of *T. molitor*, *M. domestica* and *Sitotroga calcitrans* (Hammock et al. 1974a; 1975a,c), while tissues in vitro or tissue homogenates of *M. domestica* larvae and adults, *S. bullata* adults, *S. calcitrans* adults, *Trichoplusia ni* larvae, *M. sexta* larvae and *T. molitor* larvae and pupae convert R-20458 to its corresponding diol in varying amounts (Hammock et al., 1974a; 1975a,c; Novock et al., 1975). Epoxide hydrolase activity varies from quite low in adult...
in insects or mammals, although some of the pathways are involved in environmental
degradation (Ajani and Riddiford, 1973; Hoffmann et al., 1973; Gill et al., 1974;
Hammock et al., 1974a,b). Glutathione conjugation is a well-known pathway of
epoxide metabolism in mammals, but it is apparently insignificant in R-20458 meta-
bolism in mammals and insects, possibly due to the trihalogenated nature of the
epoxide (Gill et al., 1974). Apparent conversion of an epoxide to an olefin was
reported in rats by Hoffmann et al. (1973) and this pathway has been recently
confirmed in steers (Ivie et al., 1975) apparently mediated by microorganisms in
the rumen (Ivie, 1975). Epoxide reduction has been found also in the gut contents
of mice, rats, and man but not in those of Cremophorhina portentosae (Madagascar
cockroach). Such a conversion of an epoxide to an olefin has not been reported
in insects although in T. molitor unidentified metabolites of a higher RF than
the starting epoxide have been noted on TLC (Hammock, unpublished).

Epoxides are hydrated in insects by hydratases which are membrane-bound for
the cyclodienes, NH's and juvenoids so far examined as substrates. Most activity
is in the microsomal fraction, but some activity is associated with cellular
debris and the mitochondrial fraction. It is not known if the activity in these
two fractions is intrinsic or an artifact due to contamination (Brooks et al.,
1970; Hammock et al., 1974a, 1975b; Slade et al., 1975). With most substrates, mammalian epoxide hydratase activity is also membrane-bound with the exception of
a highly active soluble fraction in liver and kidney with NH or juvenoid sub-
strates (Gill et al., 1972, 1974; Hammock et al., 1974c). Possibly the soluble
epoxide hydratase in mammals is involved in degrading metabolic by-products from
steroid biosynthesis, and insects, which do not synthesize steroids, have no need
for such enzymes.

Using the cyclodienes epoxide, HEOM, Slade et al. (1975) report a pH optimum
of 9.0 for Calliphora erythrocephala and Prodenia eridania, and 8.1 for G.
portentosae microsomal epoxide hydratases. Slade et al. (1975) also noticed a
E. bullata to very high in larval T. ni homogenates, although undoubtedly much of this variation exists due to the developmental stage of the insects examined and methods of homogenate preparation. An intensive study of the nature and properties of the epoxide hydrolases of several insects is needed to better understand their role in JH and juvencid metabolism. Such a study has probably not been completed due to the difficulties encountered in working with membrane-bound enzymes and the lack of rapid assays for epoxide hydrolase activity.

The epoxide hydrolases are thus important in the degradation of JH and epoxide containing juvenoids. Extending the alkyl branches of the epoxide moiety may serve to stabilize the epoxide by interfering with its fit at the hypothetical active sites of hydrolases. The tetrasubstituted epoxide (Ro-8-4314) (5, Fig. 2) is more stable to housefly epoxide hydrolase than R-20458 or JH I indicating that the addition of the methyl group at C-10 may sterically prevent binding at the enzyme active site or interfere with the hydration mechanism. Instead of being stabilized, the epoxide has been either eliminated or replaced with other functional groups such as the alkoxyl moiety in some juvenoids.

Metabolism of Alkoxides

The use of the alkoxyl moiety to replace the epoxide was suggested by Sorm (1971) and has led to several highly potent juvenoids in which a conformation alkoxide probably mimics the epoxide. Since the alkoxides are not highly strained ethers they are not subject to acid or base-catalyzed hydrolysis or enzymatic hydration, yet they are subject to enzymatic O-demethylation. Solomon and Metcalf (1974) found that methoprene is O-demethylated by O. fasciatus and T. molitor (Fig. 3) and that this reaction is inhibited by piperonyl butoxide. Since the tertiary alcohol produced upon O-demethylation of methoprene is more active in a few insects than the alkoxide, O-dealkylation can sometimes be considered an activation reaction. Yu and Terriere (1975) provided circumstantial evidence for
their hypothesis of in vitro O-demethylation of methoprene in *M. domestica*. Quevedo et al. (1975a) established that the tertiary alcohol is a primary metab-
olite of the mosquito larvae *Aedes aegypti* and *Culex pipiens quinquefasciatus* and the larvae of *M. domestica*. Hammock et al. (1975a) have shown that both the methoxide and ethoxide of a geranyl phenyl ether (12, Fig. 2) are metabolized by O-dealkylation in *T. molitor* larvae and adults of *S. calcitrans* and *M. domestica*. As expected Hammock et al. (1975a) found that the O-dealkylation is predominantly a NADPH dependent microsomal reaction in *S. calcitrans* and *M. domestica* and that ethoxides are dealkylated more slowly than methoxides both in vitro and in vivo.

Conjugation of the Terminal Functionality

Polar conjugates of JH and juvenoids have not been thoroughly investigated and in most metabolism studies a mixture of enzymes is used to cleave the conjugates before analysis. Slade and Wilkinson (1974) present data that in *F. sridania* in vivo and in vitro reaction of the diol functionality of JH diol or JH acid diol with sulfate is the predominate conjugation pathway. This suggests that the diol of epoxides or the tertiary alcohol of alkoxides may be similarly conjugated, but sulfate conjugation will usually occur preferentially at secondary rather than tertiary alcohols. Only the aglycone portions of other conjugates have been described.

Metabolism of Olefinic Isopropylidenes

A possibility for avoiding the epoxide moiety in juvenoids is to treat insects with the precursor olefin assuming that the insect will epoxidize the olefin to a more active compound (Jacobson et al., 1972). This conversion is difficult to show in vivo in insects since very minor conversion could account for a large increase in activity and the epoxide is labile to metabolism. The conversion of
the R-20458 diene to R-20458 (1 to 2, Fig. 4) has been demonstrated as a RAPDH-dependent reaction in *M. domestica* microsomes; however, the major metabolic pathways are totally different than those involved in R-20458 metabolism. The diene is probably oxidized on a methyl alpha to the 6,7-double bond to give an alcohol, aldehyde and acid at a very high rate, however, this rapid degradation of the diene was not observed in enzymes from *E. bullata, P. americana*, or *M. sexta*. The diene is converted cleanly to its corresponding epoxide by corpora allata homogenates from *M. sexta* and *Blaberus giganteus* (cockroach) when RAPDH is present (Hammond and Gilbert, 1974), and in this conversion the juvenoid is probably mimicking a JH precursor.

**Metabolism of the Terpenoid Chain**

Although the functional groups most susceptible to metabolic modification are usually at the extremities of the juvenoid molecule, several metabolic pathways involve the terpenoid chain itself.

**Olefinic Isomerization**

Just as in JH I (Schwiter-Peyer, 1973), a 2,3 double bond is essential for high insecticidal activity in the dienoate methoprene (Henrick et al., 1973, 1975). Quistad et al. (1975a) have shown that the 2,3 double bond of methoprene is susceptible to biological isomerization by mosquitoes and houseflies (Fig. 3). Primary metabolites and recovered methoprene had been substantially isomerized at C-2 to the 2,3 isomer which is less active by 100-fold on *A. aegypti*. Hence, economic biological activity was essentially eliminated by olefinic isomerization, and the mechanism for this provocative transformation remains unknown. The same authors showed that although methoprene was isomerized by *M. domestica* larvae to the 2,3 isomer, the converse reaction for formation of methoprene from the 2,3 isomer did not occur. The findings of Quistad et al. (1975a) have been confirmed in three strains of *M. domestica* by Hammond et al. (1975c), and one wonders if this olefinic
isomerization is involved in JH metabolism in the Diptera. Such isomerization has not been reported in the metabolism of other juvenoids or JH, probably because the investigators lacked the techniques to easily quantitate the isomers.

Olefinit Oxidation

Oxidative scission of the 4,5 double bond of methoprene by *N. domestica* results in 7-methoxycitronellal (5; Fig. 3) which is evolved as a volatile metabolite (Quistad et al., 1975a). Further oxidation and O-dealkylation of this aldehyde results in 7-methoxycitronelic acid and 7-hydroxycitronelic acid (6,7 respectively; Fig. 3) which have been detected as methoprene metabolites in *N. domestica*, *Culex pipiens*, and *Aedes aegypti*. This oxidative scission is apparently less important in insects than in other degradative systems such as plants (Quistad et al., 1974a).

An important mammalian degradation pathway for methoprene involves exhaustive catabolism of the terpeneid chain to $^{14}$C acetate which is then reincorporated into a plethora of natural products (Quistad et al., 1974b, 1975b,c). Primary metabolites from methoprene in mammals are found only in excrement while the radio-labeled residues in tissues consist mostly of biochemistry such as $^{14}$C fatty acids. The metabolic capability of insects appears to be quite different for methoprene since only primary metabolites could be characterized and degradation of methoprene to $^{14}$C acetate by insects apparently did not occur as evidenced by lack of radio-labeled natural products and the small amounts of $^{14}$CO$_2$ detected. It is likely that the geranyl moiety of geranyl phenyl ether juvenoids undergoes similar metabolism following cleavage, but such findings have not been reported because the available radio-labels are lost from the geranyl moiety following ether cleavage.

Oxidation of the isopropylidene olefin to the corresponding epoxide has already been discussed with regard to activating some juvenoids and as a potential step in the biosynthesis of juvenile hormone. The 2,3-olefinic bond in the
geraniol derived juvenoids and the 6,7-olefinic bond in farnesol derived materials are much less susceptible to chemical and biological oxidation than is the isopro-
pylidene group. The diepoxide of JH has been reported as a possible metabolite of P. malanagaster although the investigators admitted their lack of definitive evidence (Ajami and Riddiford, 1973) (5, Fig. 1). The diepoxide of R-20458 (3, Fig. 5) is a minor metabolite in microsomal systems from M. domestica abdomens and P. americana fat body (Hammock et al., 1974a), while in mammalian microsomes the diepoxide is a more important NADPH-dependent metabolite than in insects (Gill et al., 1974). Housefly microsomes with NADPH are also able to convert JH I to small amounts of the corresponding 6,7-10,11-diepoxide (Hammock, unpublished results). Although the 4,5-epoxide of methoprene is a known photoprodut (Quistad et al., 1975d), neither the epoxide nor its corresponding diol could be found as an in vivo metabolite in A. aegypti, C. pipiens or M. domestica.

Since a monoepoxide is readily converted to its corresponding diol by chemical or enzymatic hydration, it was assumed that a diepoxide would be hydrated to its corresponding tetrol (2, Fig. 4). This assumption led to several mistakes (Gill et al., 1977; Ajami and Riddiford, 1973), because the diol epoxides resulting from the hydration of one epoxide of a diepoxide rapidly cyclize to form a variety of products (Hammock et al., 1974a,b). The most abundant products are the corresponding cis and trans tetrahydrofuran diols shown to be in vitro metab-
olites of R-20458 in M. domestica (4, Fig. 4). The corresponding tetrahydrofuran diols of JH I were not detected in M. sexta tissues in vitro (Hammock et al., 1975b) but are minor metabolites of JH I in housefly microsomes. An olefinic diol when epoxided either chemically or enzymatically also undergoes cyclization to yield tetrahydrofuran diols and such reactions have been found in vitro in several insects (Hammock et al., 1974a). The question then arises if tetraols are ever formed from compounds of the general structure 1,2-epoxy-5-hexene.

Although very minor metabolites of t-20458 in M. domestica microsomes do
co-chromatograph with the tetraol of R-20458, 2,3,6,7-tetraols were disallowed by derivatization and co-crystallization studies (Hammock et al., 1974a). In mammals such tetraols occur from juvenile metabolism in vivo in addition to tetrahydrofuran diols possibly arising from a coupled oxygenase-hydrolase system (Hoffman et al., 1973; Gill et al., 1974).

Other Metabolism of the Terpenoid Chain

Metabolic reduction of the 2,3-double bond in geraniol has been shown in mammals (Kohn et al., 1936). Thus, reduced metabolites of geranyl phenyl ethers such as R-20458 might be expected, but none have been reported. Methoprene is reduced to totally saturated hydroxy and methoxy acids by chickens (but not insects) which are then largely stored by glycerides (Quistad et al., 1975a). Other metabolic pathways for the terpenoid chain have also not been established in insects. The phenols from mammalian metabolism of geranyl phenyl ethers probably result from rearrangement of an unstable intermediate which has been hydroxylated on carbon 1 of the terpenoid side chain. Hydroxylations on carbons alpha to olefins are common degradative reactions and such hydroxylated metabolites of R-20458 have been identified on the basis of me in the rat (Hoffmann et al., 1973). Such hydroxylated compounds have not been identified as metabolic products from any JH or juvenile in insects, but they probably account, in part, for minor metabolites often found during degradation in juvenoids by some insects.

JUVENOID MODIFICATION OF JH METABOLISM

It has been suggested (Slade and Wilkinson, 1973) that juvenoids possess minimal inherent juvenile hormone activity but rather are morphogenetically disruptive because they inhibit the JH degradation system. Hence JH is synergistically protected from degradation and remains at a high titer in the relevant tissues.
The generalization that juvénoid activity is synergistic rather than intrinsically hormonal has been refuted by several papers (e.g. Solomon and Walker, 1974), yet Slade and Wilkinson's paper did acknowledge some interesting problems. For instance, Slade and Wilkinson (1973) refer to propyl 2-propynylphosphonate (NIA 16388) as a juvenile hormone analog (JHA) due to its activity in a morphogenetic assay (Bowers, 1968) while a more restrictive definition of juvenile hormone mimic or juvénoid requires that the compound restores a normal JH mediated function in an allatectomized insect. However, Solomon et al. (1973) have shown that at least piperonyl butoxide has low inherent JH activity. Data indicating that juvenoids can stabilize JH at physiological levels is lacking. One can, however, easily predict that some juvenoids such as the methylenedioxyphenyl ethers will act as microsomal oxidase inhibitors and possibly retard their own metabolism (Brooks, 1973), and the high doses of some known synergists required to produce a morphogenetic response may actually stabilize intrinsic JH (among other actions). Also interesting is the low but detectable inhibition of insect epoxide hydratase by some juvenoids (Brooks, 1973; Slade et al., 1975).

The structural similarity of most juvenoids to JH suggests that juvenoids may mimic JH in several systems and that competitive metabolism is quite probable. Undoubtedly there are many sites of action for JH in an insect and each site might be slightly different; a given compound may be a JH mimic, antagonist, or have no effect at the various active sites. Part of the remarkably disruptive activity of some juvenoids may result from their mimicking the natural hormone at some, but not all of its sites of action.

Terriere and Yu (1973) and Yu and Terriere (1974a) reported that high doses of some juvenoids and synergists modify xenobiotic metabolism in M. domestica adults. Of more interest were the findings by these workers (Yu and Terriere, 1974b) of substantial changes in the microsomal oxidase levels of M. domestica during development and that these changes may be, in part, under the control of JH and thus influenced by juvenoids.
METABOLISM ACTIVITY CORRELATIONS

Both activation and inactivation of virtually every class of pesticides determines their selective toxicity to different organisms or various stages of the same organism. The great differences in juvenoid activity suggest that JH receptors vary greatly in their requirements for juvenoid fit at the active site(s). Since receptors have not been characterized, we cannot sharply distinguish between selectivity caused by metabolism and action at the active site.

Hannock et al. (1974b, 1975a) suggested that a refractory pool (possibly specific carrier molecules) may be important in explaining juvenoid action, and this subject is examined in several papers presented in this book. However, assuming equal response at the active sites, some correlations can be drawn between metabolism and activity.

Using parabiosis experiments Reddy and Krishnakumar (1972) presented evidence that several juvenoids were more stable and more active in T. molitor than JH I. Quistad et al. (1975a) explored the age-dependent response by A. aegypti mosquitoes to methoprene. These mosquito larvae are known to be more sensitive to methoprene as late (24-48 hr) fourth instars. Contrary to expectations, the total amount of methoprene metabolized by A. aegypti increased with age in third, early fourth (2-24 hr), and late fourth instars and activated metabolites were contraindicated by the decreased activity of known metabolites. However, other factors such as selective cuticle penetration may have been operative since the actual amount of unmetabolized methoprene inside the most sensitive late fourth-instar larvae was 2-3 times greater than in less susceptible younger larvae. The same workers also found that Culex larvae are apparently less sensitive to methoprene than A. aegypti because they inactivate it faster.

In a comparative study using alkoxides of geranyl phenyl ethers (10, Fig. 2) Hannock et al. (1975a) suggested that the high activity of ethyl and propyl
alkoxides on *T. molitor* is due in part to the enhanced stability of the alkoxides over the epoxides. The same authors also warned that in insects with high levels of 0-dealkylating enzymes but low levels of epoxide hydratases as *M. domestica* or *S. caltira*, the opposite relationship between structure and activity might be found.

In most cases metabolism of juvenoids results in products with considerably reduced morphogenic activity. A possible exception to this rule may be 0-dealkylation of methoprene to the hydroxy ester which appears to be an activated metabolite in *O. fasciatus* (Solomon and Metcalf, 1974) and is inherently more active against some insects under bioassay conditions (Henrick C. A. and Staal, G. B., unpublished). Another exception is the diene of R-20458 which has already been discussed as a probable metabolic precursor of the more potent R-20458 (Hammock et al., 1974a).

**Juvenoid Metabolism as a Resistance Mechanism**

The development of resistance to a pest control agent by the target species is a major problem in insect control. Some workers predicted that juvenoids would be immune to the problem to resistance although most entomologists feel that resistance can develop to any agent provided there is intense selection pressure and an adequate genetic pool. Thus, it was not surprising when cross-resistance between juvenoids and insecticides was found (Dyce, 1972; Cerf and Georghiou, 1972, 1974; Jenskin and Vinson, 1973; Flapp and Vinson, 1973; Vinson and Flapp, 1974, Kadri, 1975). Laboratory selection pressure with juvenoids has resulted in the development of resistance in several insect species (Brown and Brown, 1974; Brown et al., 1974; Georghiou et al., 1974; Georghiou, 1975). Although there are numerous mechanisms of resistance to insecticides, it has been noted that strains of *M. domestica* with high cross-resistance to juvenoids have high microsomal oxidase levels (Flapp and Vinson, 1973; Cerf and Georghiou, 1974; Vinson and Flapp, 1974). Terriere and Yu (1973) indicated the involvement
of the microsomal oxidase system in the degradation of methoprene by *M. domestica* enzymes and further showed that the degradation was more rapid in enzymes from resistant strains. Hammock *et al.* (1974a) found that R-20458 is more rapidly metabolized (a susceptible SCR strain) in enzymes from a R-Bagon (propoxur) strain than a susceptible SCR strain. The R-Bagon strain shows cross-resistance to several juvenoids and has a high microsomal oxidase level (Vinson and Plapp, 1974). The R-Dimethoate strain of *M. domestica* shows a high degree of cross-resistance to juvenoids (40X), and it and other strains have been further selected with methoprene to obtain highly resistant strains (R-methoprene, 1190X; Georgiou, 1975). Both the R-Dimethoate and R-methoprene strains show a high level of microsomal oxidase. Larvae, pupae, and adults of these strains are capable of rapidly metabolizing methoprene and R-20458 both *in vitro* and *in vivo* with O-demethylation and α-hydroxylation being the predominant respective pathways. Although the *in vitro* metabolism of methoprene by R-Dimethoate and R-methoprene microsomes is similar, a slight decrease in penetration coupled with a slight increase in *in vivo* metabolism in white larvae results in a lower peak level of methoprene in larvae of the R-methoprene strain when low topical dose levels are used. No differences in epoxide hydratase or esterase activity were detected in any of the strains. It is unlikely that a complete explanation has been found for the resistance present in the R-methoprene strain (Hammock *et al.*, 1975b), but possibly a narrow window of susceptibility accounts in part for the shallow slope of the dose-response regression lines noted by Georgiou (1975) or the resistance may be due in part to the R-methoprene strain no longer recognizing methoprene as a JH mimic.

It is obvious that insects can become resistant to juvenoids and indeed that cross-resistance already exists. Whether resistance will become a problem depends upon the intensity of selection pressure caused by juvenoid use. Laboratory work with mosquitoes is encouraging in that only minimal resistance has
induced under intense selection pressure (Georgiiev et al., 1976); however, studies with *Microtus domestica* may be indicative of future resistance problems. It is likely that enhanced metabolism of juvenoids will be an important factor in resistance.
Figure Legends

Fig. 1. Established pathways of JH metabolism in insects. Conjugation of the diol and acid diol also may occur.

Fig. 2. Structures of juvenoids involved in metabolism studies. Compounds 1, 4, 6, 7, 8, 9 are from Zoecen Corporation; 2 from Stauffer Chemical Company; and 5 from Hoffman La Roche.

Fig. 3. Major nonconjugative pathways of methoprene metabolism in insects.

Fig. 4. Major routes of R-20458 metabolism in insects. Metabolism of both the geranyl and phenyl moieties can occur on the same molecule. Dotted lines indicate metabolic routes not established in insects.
References


