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CHAPTER 1

Metabolism and mode of action of juvenile hormone, juvenoids, and other insect growth regulators

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INTRODUCTION

The insect growth regulator field emerged with the elucidation of the structure of juvenile hormone (JH). Based on the early work of Wigglesworth (1936, 1970) and other endocrinologists, the interesting report by Williams (1956) of high levels of JH activity in the abdomens of adult male *Hyalophora cecropia* moths, and the synthesis of methyl-10,11-epoxyfarnesoate by Bowers *et al.* (1965), the structure of JH I (Figure 1.1) was determined by Röller *et al.* (1967). Subsequently, two other JH homologues were identified (Figure 1.1) (Meyer *et al.*, 1970; Judy *et al.*, 1973) (for reviews see Wigglesworth, 1970; Menn and Beroza, 1972; Gilbert, 1976; Schooley, 1977). Williams (1967) predicted the advent of a new generation of pest control agents based on these insect hormones. As reviewed by Slamá *et al.* (1974), subsequent research produced numerous JH mimics or juvenoids, some more active and more stable than the natural hormones. The juvenoids with another group of compounds, the benzoylphenyl urea chitin synthesis inhibitors (Wellinga *et al.*, 1973), introduced a new group of insecticides termed insect growth regulators (IGRs). In this review, an overview of the mechanism of action of the insect growth regulators will be presented. The environmental fate and the metabolism of the IGRs by target and non-target organisms will be reviewed in greater detail and the promise of current and future IGRs discussed. A discussion of IGR metabolism can hardly be undertaken without a feeling for the metabolism of JH itself; thus, an earlier review of juvenoid and JH metabolism will be updated (Hammock and Quistad, 1976) and expanded to include other IGRs.

Such attention to the routes of IGR degradation is warranted because these compounds represent new structures for the metabolism chemist. For instance, several interesting routes and concepts in xenobiotic metabolism surfaced from the study of juvenoids. The Hoffman *et al.* (1973) report of a Baeyer–Villiger-like oxidation has not been verified *in vitro*, but it presents interesting mechanistic considerations. The report of Quistad *et al.* (1974b) that a pesticide can be metabolized and then converted into natural products certainly illustrates that retention of tissue radioactivity following administration of a xenobiotic should not automatically be considered as a sign of biostability. The elucidation of epoxide hydration as a major route of insect metabolism of epoxide-containing juvenoids (Gill *et al* 1972) coincided with the analogous discovery of epoxide

hydration and ester hydrolysis being major routes of natural juvenile hormone metabolism (Slade and Zibitt, 1971, 1972). The significance of epoxide reduction initially detected in rats (Hoffman *et al.*, 1973), and clearly shown to occur in the rumen contents of steers and sheep (Ivie *et al.*, 1976; Ivie, 1976), seems to occur in the gut contents of a variety of mammals, including man. This route of metabolism, known from other organisms (Yamamoto and Higashi, 1978), surely warrants further investigation and may be important in the inactivation of toxins, mutagens, and carcinogens in the gut (Ivie, 1976; Callen, 1978). In spite of the early reports of Gill *et al.* (1972, 1974) to the contrary, it had been widely assumed that all epoxide hydrolase activity was membrane bound. Subsequent reports certainly demonstrate that for terpenoid epoxides high hydrolase activity is in the cytosolic or soluble fraction (Hammock *et al.*, 1976; Mumby and Hammock, 1979a, b, c) as well as the mitochondrial fraction (Gill and Hammock, unpublished). They explain how the activity has been overlooked in many laboratories for a decade (Hammock *et al.*, 1980a, b; Ota and Hammock, 1980) and demonstrate that the cytosolic enzyme hydrates a wide variety of substrates including mutagens and suspect carcinogens (El-Tantawy and Hammock, 1980; Hasegawa and Hammock, unpublished). The investigation of precocene metabolism may help to explain the mechanism of action of these interesting compounds as well as the intracacies of JH biosynthesis (Jennings and Ottridge, 1979; Soderlund *et al.*, 1980).

The IGRs represent an exciting concept in the development of new insecticides. The investigation of their metabolism and action should continue to provide insights useful for improving IGR structures. Hopefully, such improved

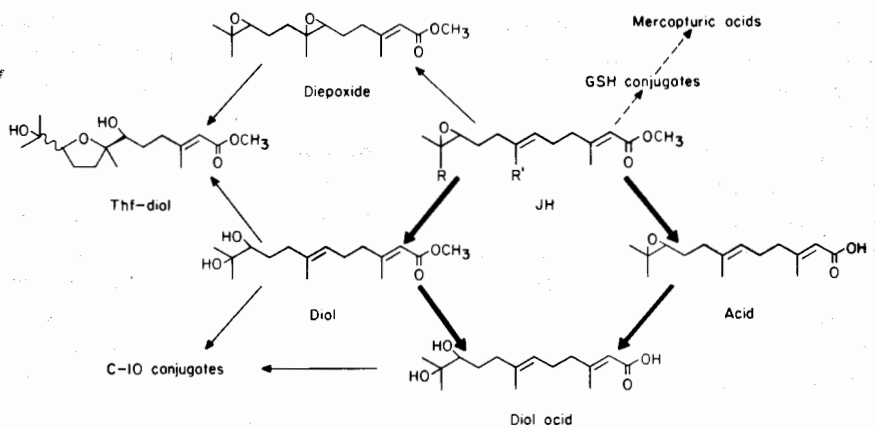


Figure 1.1 Metabolism of juvenile hormone (JH). Metabolites are shown for JH III ($R = R' = CH_3$). The structures of JH I ($R = R' = (C_2H_5)$ and JH II ($R = C_2H_5$, $R' = CH_3$) are similar. Heavy lines indicate the major pathways of metabolism shown to occur in most insects. GSH conjugation (dashed line) has not yet been demonstrated in insects

compounds will be even more compatible with environmentally sound integrated pest management programmes.

JUVENOIDS—MODE OF ACTION

Numerous publications have appeared over the last decade concerning the mode of action of insect juvenile hormones. Such studies can be conveniently divided into the following chronological categories.

(1) Synthesis (including its initiation and regulation) in the corpora allata.
(2) Transport of JH in haemolymph (binding proteins).
(3) Degradation.
(4) Cellular targets for expression (epidermis, fat body, prothoracic gland, etc.).
(5) Macromolecular events (synthesis of protein, RNA, or DNA, and enzyme regulation).
(6) Overt morphological effects.

The synthesis and transport of juvenile hormones in two insect species have been compared by Kramer and Law (1980) while the morphological results of juvenile hormone activity in insects have also been summarized (Staal, 1975; Sehnal, 1976). A comprehensive compilation of data relating to the mode of action of juvenile hormones was represented by Gilbert (1976) and Gilbert and King (1973). In analysing information concerning the mode of action of juvenile hormones, it is readily apparent that many uncertainties complicate the issue. A unified explanation of insect JH mode of action is still non-existent, but rather in its place are numerous, often unrelated facts from a multitude of different researchers and different insects. In this review we will address the question of *juvenoid mode of action* rather than JH mode of action. However, although considered separately, the modes of action of juvenoids and JH are likely to be synonymous. Indeed, there are many examples where juvenoids can serve as direct replacements for JH.

In overview, there are two likely methods by which juvenoids disrupt insect development. In one method juvenoids may act as perfect mimics of JH. Their disruptive effect stems from the presence of a relatively large amount of juvenoid, overpowering the homeostatic mechanisms present in the insect or from their presence at inopportune times. A second mechanism may involve juvenoids acting as imperfect mimics of JH. In this case, the juvenoid may be a potent JH mimic at some sites but a poor mimic or even antagonist at other sites leading to a disruption of insect development.

Effects on JH Degradation

Slade and Wilkinson (1973) proposed a rather controversial explanation of the mode of action of juvenoids in insects. They claimed that rather than being intrinsically hormonal, juvenoids were synergistic in preventing the degradation

of natural JHs. This proposal was supported by data showing the apparent stabilization of JH I *in vitro* when midgut preparations from *Spodoptera eridania* (southern armyworm) were exposed to several juvenoids (including methoprene and hydroprene). Slade *et al.* (1975) and Brooks (1973) showed that hydration of an epoxide of a cyclodiene (HEOM) was also inhibited by several juvenoids (including R-20458 and methoprene). These authors suggest that inhibitors of cyclodiene epoxide hydrolase may also inhibit JH epoxide hydrolase. Hence, these juvenoids are potential synergists of natural JHs. In conflict with the above conclusions were data from Terriere and Yu (1973, 1974) using *M. domestica* which showed not inhibition of cyclodiene epoxide hydrolase (using heptachlor epoxide), but rather either no effect (hydroprene) or slight enhancement of epoxide hydrolase activity (methoprene). Downer *et al.* (1975) offered further support for the Slade–Wilkinson hypothesis of a synergistic mode of action by showing that esterases of *Aedes* were inhibited by methoprene. However, the conclusions of Downer *et al.* (1975) are suspect since they used α-naphthylacetate as a substrate to assay for JH esterase and α-naphthyl esters may only be reflective of general esterase activity, rather than JH-specific esterase (see Hooper, 1976).

Although under certain conditions juvenoids may exert their action (at least in part) through synergism of endogenous hormone, considerable data refute the notion of juvenoids solely as alternative substrates or inhibitors of the enzymes of JH degradation (Solomon and Walker, 1974). In the Colorado potato beetle (*Leptinotarsa decemlineata*) it has been established that JH is metabolized mainly by JH esterases (Kramer *et al.*, 1977), but two biologically active juvenoids (methoprene and hydoprene) are not substrates for this insect's JH esterase (Kramer and deKort, 1976b) and actually increase *in vivo* esterase activity for JH I (Kramer, 1978). Although pupal haemolymph from *Tenebrio molitor* contained the highest esterase activity for JH I of five insect species tested by Weirich and Wren (1976), methoprene was not hydrolysed. Methoprene is also not hydrolysed by esterases of *Culex pipiens* (Hooper, 1976), *Blaberus giganteus* (cockroach; Hammock *et al.*, 1977b), and *Manduca sexta* (Weirich and Wren, 1973) even though the compound is morphologically active on these insects. Larval esterases of *Diatraea grandiosella* (southwestern corn borer) bound neither hydroprene nor methoprene, but did bind JH I; hence, in this insect also, these juvenoids do not appear to synergize JH by inhibiting its ester hydrolysis (Brown *et al.*, 1977). Methoprene also failed to inhibit the *in vitro* hydrolysis of JH by esterases of *Trichoplusia ni* (Sparks and Hammock, 1980b), and as discussed later some juvenoids may even stimulate JH metabolism.

Effects on JH Synthesis

The actual modulation of JH III synthesis by hydroprene in *Diploptera punctata* (a viviparous cockroach) was shown elegantly by Tobe and Stay (1979).

By determination of the titre of JH III these authors demonstrated that hydroprene stimulated JH synthesis at low doses and suppressed hormone biosynthesis at high doses. It is likely that the juvenoid is acting on a normal JH receptor by feedback regulation of JH biosynthesis. Of course, since hydroprene is intrinsically gonadotrophic in this cockroach, even though natural JH III synthesis has been suppressed at high doses of juvenoid, the overt morphological response (i.e. oocyte elongation) could mistakenly suggest a high JH III titre.

These studies and those of Schooley and Bergot (1979) illustrate the interesting concept that many known juvenoids are likely to have some JH antagonist action at the physiological, if not the biochemical level. However, such antagonistic effects may be difficult to distinguish in the intact insect, but they may enhance the disruption of insect development by some juvenoids.

Effects on Site of Action

Since JHs are both morphogenetic and gonadotrophic, it is not surprising that the tissues associated with such effects would be regulated by juvenoids. Using the cabbage armyworm (*Mamestra brassicae*) Hiruma *et al.* (1978b) showed that methoprene inhibits release of neurosecretory material (i.e. prothoracicotropic hormone, PTTH) from cerebral cells, but may not affect the synthesis of new PTTH in the same cells. These results suggest that a high JH titre late in the last larval instar of *M. brassicae* inhibits the release of PTTH from the brain and the subsequent lack of PTTH decreases α-ecdysone secretion from the prothoracic gland. Hence, the cerebral neurosecretory cells appear to be regulated by both β-ecdysone and JH. In the absence of PTTH the prothoracic gland in *M. brassicae* is activated by methoprene, but only in the last part of the final instar and in the pupal stage (Hiruma *et al.*, 1978a). By means of ligature experiments Cymborowski and Stolarz (1979) demonstrated that methoprene inhibits the prothoracic glands of *Spodoptera littoralis* at the beginning of the final instar and then stimulates the glands shortly before pupation. Activation of pupal prothoracic glands by juvenoids seems to be a rather general response in lepidopterous insects since Krishnakumaran and Schneiderman (1965) showed increased prothoracotrophic activity upon treatment of brainless, diapausing pupae with farnesol, farnesyl methyl ether, farnesyl diethylamine, nerolidol, and dodecyl methyl ether. As already mentioned, Tobe and Stay (1979), using hydroprene, have shown that the biosynthetic activity of the corpus allatum itself is regulated by JH.

After secretion from the glands responsible for morphogenetic hormone production, JH is transported (in part) to the epidermis, a primary target site. By using (2*E*,6*E*)-10,11-epoxyfarnesyl propenyl ether Schmialek *et al.* (1973) reported a JH receptor in the epidermis of *Tenebrio molitor* pupae which appeared to accumulate the juvenoid against a concentration gradient. Once in the epidermis it appears (at least for *M. sexta*) that JH is hydrolysed primarily by

esterases (Mitsui *et al.*, 1979). Specific effects of methoprene on the fat body (Couble *et al.*, 1979) and ovary (Postlethwait and Gray, 1975) will be discussed subsequently.

Effect on Macromolecular Biosynthesis

Krypsin-Sorensen *et al.* (1977) found that the abnormally increased somatic growth of *Spodoptera littoralis* treated with methoprene was not associated with hypermetabolic activity (e.g. unusually large O_2 consumption and CO_2 release). Work in the same laboratory had previously revealed that juvenoid treatment of dermestid beetle larvae caused an enormous rise in total metabolic rate, but without formation of extra-larval instars (Sláma and Hodkova, 1975). The glycogen and lipid reserves were considerably depleted in *Aedes aegypti* pupae resulting from fourth instar larval treatment with methoprene (Downer *et al.*, 1976). Thus, reduced energy supplies may contribute to pupal mortality.

Since it has been suggested that insect hormones may act primarily at the genetic level, a number of investigations of juvenoid effects on nucleic acid synthesis have been reported. With imaginal wing discs of *Calliphora*, farnesol inhibits incorporation of thymidine into DNA (Vijverberg and Ginsel, 1976), while methoprene has no effect on thymidine incorporation, but does decrease inclusion of uridine into DNA (Scheller *et al.*, 1978). In the *Calliphora* wing disc assay methoprene increases rRNA synthesis and inhibits production of mRNA (Scheller *et al.*, 1978). Likewise, *de novo* synthesis of rRNA from [2-^{14}C]glycine is accelerated by methoprene in *Musca domestica* (Miller and Collins, 1975). Himeno *et al.* (1979) reported that DNA and particularly RNA synthesis was inhibited by methoprene in *Culex molestus*. Methoprene was a stronger inhibitor of RNA synthesis than either puromycin or actinomycin D, but the RNA inhibition was reversible. Both methoprene and hydroprene reduce uridine incorporation into the RNA of larval *Drosophila* (Breccia *et al.*, 1976) while the RNA of adult *D. melanogaster* treated with methoprene was 6% enriched in poly(A) sequences (Gavin and Williamson, 1978). Thus, juvenoids regulate nucleic acid synthesis with the observed effect (i.e. stimulation or repression) dependent on stage of development, type of insect, method of assay, etc.

Since genetic manipulation is often expressed as protein synthesis, it follows that protein production would also be regulated by juvenoids. Methoprene is an inhibitor of protein synthesis in larval *Drosophila* homogenates (Breccia *et al.*, 1976) and cultured *Culex* cells (Himeno *et al.*, 1979), but had no effect in a *Calliphora* wing disc assay (Scheller *et al.*, 1978). JH itself inhibits protein synthesis in imaginal discs of *D. melanogaster* (Fristrom *et al.*, 1976).

A particularly important protein is vitellogenin which is essential to insect egg maturation. Methoprene is effective in converting the fat body of adult female *Locust migratoria* from a nutrient storage depot to a site of vitellogenin synthesis and secretion (Chen *et al.*, 1976; Couble *et al.*, 1979). Methoprene activates the

fat bodies in isolated *D. melanogaster* abdomens to promote vitellogenesis, thereby promoting the maturation of oocytes (Postlethwait *et al.*, 1976; Handler and Postlethwait, 1978). Methoprene not only enhances the synthesis of vitellogenin, but also increases its sequestration from the haemolymph into the oocyte (Handler and Postlethwait, 1978). The stimulation of yolk protein synthesis in *D. melanogaster* has been attributed to production of poly(A)-containing RNA (Gavin and Williamson, 1978). Methoprene also regulates the acid phosphatase activity in the ovary of *D. melanogaster* and this enzyme is necessary for yolk metabolism (Postlethwait and Gray, 1975).

Thus, in summary, juvenoids regulate the activity of insect glands associated with growth hormone production. The type of effect on these glands (neurosecretory cells, prothoracic gland, corpus allatum) is largely dependent on the timing of application relative to the stage of metamorphosis. The stimulation and inhibition of nucleic acid or protein synthesis are equally a function of the exact timing of juvenoid application. Hence, observations of gland activity or macromolecular biosynthesis are intimately controlled by metamorphosis.

Several facets of the mode of action of juvenoids in non-insect species have been examined. Both methoprene and hydroprene decrease protein synthesis in larval shrimp (Breccia *et al.*, 1977). Although JH I, II, and III are uncouplers of oxidative phosphorylation in mouse liver mitochondria, methoprene and epofenonane were 10-fold less active (Chefurka, 1978). JH I, methoprene, and triprene (ZR-619) all depress the synthesis of DNA, RNA, and protein in mouse cells, but both juvenoids were less inhibitory than JH itself (Chmurzyńska *et al.*, 1979).

RADIOSYNTHESIS OF JH AND JUVENOIDS

Several pathways have been used for the radiosynthesis of juvenoids. As with any pesticide, a ^{14}C label is useful for research on mammalian metabolism and environmental degradation because it unambiguously traces at least one carbon in the molecule. However, the extraordinary effectiveness of juvenoids leads to very low effective doses in insects. Thus, ^{14}C and even many ^{3}H labels have too low a specific activity for many mechanism of action and metabolism studies at reasonable doses.

Racemic juvenile hormone I (JH I, Figure 1.1) (25 mCi mmol^{-1}) was first labelled in the 2-position by an Emmons modification of the Wittig reaction using trimethyl [2-^{14}C]phosphonoacetate prepared from methyl [2-^{14}C]bromoacetate (Hafferl *et al.*, 1971). The first commercially available JH I was prepared by New England Nuclear Corporation by selective reduction of an alkyne to an ethyl branch at C-7 of the *E,E,cis* precursor. In later years, this product has been replaced by JH I and now JH III labelled at C-10 by sodium borotritide reduction of the corresponding haloketone to the *erythro*- and *threo*-diastereomers of the resulting halohydrins. Following chromatographic separa-

tion, the *threo* isomer was cyclized in base to yield *E,E,cis*-JH I or *E,E*-JH III ($\sim 10\,Ci\,mmol^{-1}$) (Ahern and Schooley, unpublished).

The lability of the methyl ester of juvenile hormone limits the usefulness of such methyl-labelled preparations; however, the production of methanol from the hydrolysis of JH forms the basis of a number of rapid methods for the assay of JH esterase activity (Sanburg *et al.*, 1975a; Nowock *et al.*, 1976; Hammock *et al.*, 1977b; Vince and Gilbert, 1977). The availabiity of potent JH esterase inhibitors may also extend the usefulness of such preparations (Hammock *et al.*, 1977b; Sparks and Hammock, 1980a, b). Sanburg *et al.* (1975a) prepared methyl-labelled JH I by sodium methoxide catalysed transesterification with [^{3}H]methanol to yield a product with a specific activity of $\sim 8.3\,mCi\,mol^{-1}$. Trautmann *et al.* (1974) and Hammock *et al.* (1977b) prepared labels of 4.3 and $2.5\,mCi\,mol^{-1}$, respectively, by exchanging the acid proton of JH acid with T_2O and exposing the acid to diazomethane. This method was based on the earlier work by Trautmann (1972) on JH III and a dichloro analogue labelled at 0.49 and $3.7\,Ci\,mmol^{-1}$, respectively. Still higher activities could theoretically be obtained by the alternate pathway of using tritiated diazomethane (Denmore and Davidson, 1959). Hammock *et al.* (1977b) reported that diazomethane may have reacted with the terpenoid chain of JH and that this byproduct(s) was only detected by high pressure liquid chromatography. Such side reactions should not present problems if lower specific activities for metabolism studies are required. Peter *et al.* (1979a) demonstrated a biochemical method for preparing [*methyl*-^{14}C]JH I based on the methylation of JH acid catalysed by homogenates of the sex accessory glands of male *Hyalophora cecropia* (Shirk *et al.*, 1976; Weirich and Culver, 1979). The methyl donor, carrier-free *S*-[*methyl*-^{3}H]-adenosyl-L-methionine, is commercially available and remarkable yields of >90% ^{3}H incorporation into JH I have been reported. The method uses expensive starting materials, but an optically active product with very high specific activity can be obtained in small batches. Modifications of this procedure could yield labelled JH at a reasonable price to biological laboratories.

For conjugated systems such as the juvenile hormones, metal-catalysed proton exchange is seemingly an attractive route of radiosynthesis. When *E,E*-farnesoic acid was heated in a dimethylformamide/D_2O mixture with an activated-platinum catalyst, high deuterium incorporation was obtained. G.l.c.-m.s. and n.m.r. analysis indicated that >35% of the material was the *E,E* isomer following the reaction and that the other mass could be largely accounted for by isomerization at the 2*E* and 6*E* positions. The majority of the deuterium was at the C-3 methyl group with moderate exchange at other allylic positions and some exchange of the olefinic C-2 proton itself. An analogous reaction with carrier free T_2O resulted in a product of the correct polarity, but much of the product apparently lacked the 10,11-olefin. Following reaction with diazomethane, only a low yield of *E,E* methylfarnesoate ($8.4\,Ci\,mmol^{-1}$) was obtained (Hafferl, Schooley, and Hammock, unpublished; Hammock, 1975). D_2O and low specific

activity T_2O reactions with methoprene and methoprene acid indicate even more facile incorporation in these dienoate molecules which also lack the labile 10,11 olefin.

It is very likely that JH mimics of high specific activity could be useful, perhaps even more useful than the natural hormone, in mechanism of action studies. The utility of such compounds can even be further enhanced if they are refractory to metabolism. Such an approach was demonstrated by Schmialek *et al.* (1976) by the synthesis of (2*E*, 6*E*)-10,11-epoxyfarnesyl[2,3-^{3}H]propenyl ether from the corresponding propargyl ether by reduction with tritium gas catalysed by quinoline (chinoline) poisoned Lindlar catalyst.

The radiosynthesis of [10-^{3}H]methoprene involved methoxymercuration of the olefinic precursor with mercuric acetate in methanol (Brown and Geoghegan, 1970; Henrick *et al.*, 1973) followed by conversion of the resulting organomercurial acetate to its chloride. The organomercurial chloride was reduced with sodium borotritide to yield an isomer mixture of [10-^{3}H]methoprene with a specific activity of >1.9 Ci mmol^{-1} (Schooley *et al.*, 1975a) (Figure 1.2).

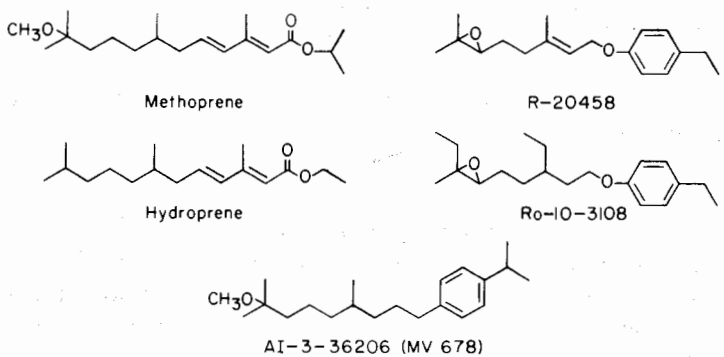


Figure 1.2 Representative juvenoid structures. Methoprene and hydroprene are referred to as dodecadienoates (Henrick *et al.*, 1973), R-20458 (Pallos *et al.*, 1971) and Ro-10-3108 are referred to as terpenoid phenoxy ethers, and AI-3-36206 (Hangartner *et al.*, 1976) is referred to as an arylterpenoid (Schwarz *et al.*, 1974)

Synthesis of [5-^{14}C]methoprene involved the carbonation of the Grignard of 1-bromo-2,6-dimethyl-5-heptene with $^{14}CO_2$ and subsequent conversion to [1-^{14}C]citronellal. Condensation with di-isopropyl 3-isopropoxycarbonyl-2-methyl-2-propenyl phosphonate (Henrick *et al.*, 1973) yielded [5-^{14}C]isopropyl (4*E*)-3,7,11-trimethyl-2,4,10-dodecatrienoate which could be reduced to hydroprene (Bergot and Schooley, unpublished) (Figure 1.2) or subjected to methoxymercuration-reductive demercuration to yield [5-^{14}C]methoprene with a specific activity of 58 Ci mmol^{-1} (Schooley *et al.*, 1975a). Hydroprene, methyl epoxyfarnesoate, ethyl dichlorofarnesoate, and R-20458 were prepared with specific activities in the 50 mCi mmol^{-1} range by tritiation (T_2O) of intermediate aldehydes (Ajami and Crouse, 1975).

Several syntheses of the juvenoid R-20458 (Figure 1.2) have been reported with a label in both the geranyl and ethylphenoxy portions of the molecule. The reduction of citral (a geranial/neral or 2*E*/2*Z* mixture) with sodium borotritide to (2*E*/2*Z*) [1-^{3}H]geraniol, bromination of the alcohol and a Williamson ether synthesis with 4-ethylphenol followed by epoxidation of the 6,7-olefin resulted in [1-^{3}H]geranyl R-20458 at 33 mCi mmol^{-1} (Kamimura *et al.*, 1972). Alternatively, the aromatic protons on 4-ethylphenol were exchanged by heating with sulphuric acid in tritium water. The resulting tritiated phenol was used as above in the synthesis of R-20458 labelled at >650 mCi mmol^{-1} in the phenyl ring (Kamimura *et al.*, 1972). Kalbfeld *et al.* (1973) synthesized [*phenyl*-^{14}C]R-20458 at 17 mCi mmol^{-1} by a similar procedure using a Williamson ether synthesis to couple the labelled phenol with 6,7-epoxygeranyl bromide. All three radiolabels proved useful in metabolism studies with several limitations. Ether cleavage immediately resulted in the loss of half of the [*geranyl*-1-^{14}C] label and oxidation of the resulting aldehyde, undoubtedly, resulted in total loss of the label. The ^{3}H-ring label was suitable for *in vitro* work, but apparently acidic conditions in the stomach caused some exchange of the labile aromatic protons. The ^{14}C label has proven very useful for metabolism studies, but following ether cleavage, it fails to trace the interesting geranyl portion of the molecule, and its specific activity is too low for most biological research.

The closely related juvenoid, Ro-10-3108 (Figure 1.2), was labelled in the 2,3-position by exposure to tritium gas in the presence of platinum. A product of ~2.3 Ci mmol^{-1} was obtained with reduction of the epoxide apparently not a problem (Dorn *et al.* 1976). The 7-methoxy and 7-ethoxy analogues (Figure 1.2) of R-20458 were prepared from the corresponding ^{3}H-ring labelled diene described above by solvomercuration–demercuration in methanol or ethanol to yield products labelled at >600 mCi mmol^{-1} (Hammock *et al.*, 1975a).

Future work in the area of radiosynthesis will probably lead to the very high specific activities usually needed for hormone receptor studies. In this research, juvenoids which are biologically stable and easier to label are, in some cases, likely to be used in lieu of natural JH.

JUVENILE HORMONE METABOLISM

Major Pathways of JH Metabolism

The basic pathways of juvenile hormone metabolism were first illustrated by Slade and Zibitt (1971, 1972) and include ester hydrolysis and epoxide hydration followed by conjugation (Figure 1.1). These observations were expanded to other insects in surveys by White (1972), Ajami and Riddiford (1971, 1973), and a host of subsequent workers. The literature through 1975 on JH metabolism was reviewed by Hammock and Quistad (1976) and some subsequent work has dealt with expanding early observations to a wider variety of insects, correlations of

JH metabolism with developmental changes, and a biochemical characterization of the proteins involved. Hopefully, such work will continue to expand yielding data on the regulation of JH metabolism and allowing development of a model of JH *in vivo* kinetics. General aspects of JH metabolism in several insect orders will first be covered followed by a discussion of insect epoxide hydrolases (EH). JH esterases (JHE) will be covered in more detail including assay methods, changes during development, inhibition, and regulation. Finally, the influence of JH carriers on metabolism will be considered. For more detailed information on aspects of JH chemistry, biosynthesis, and action, numerous reviews are available (Schneider and Aubert, 1971; Menn and Beroza, 1972; Akamatsu *et al.*, 1975; Gilbert, 1972, 1974, 1976; Gilbert *et al.*, 1976, 1977, 1978; Riddiford and Truman, 1978; Kramer and Law, 1980; deKort, 1981). The metabolism of JH in relation to insecticide resistance has been reviewed by Sparks and Hammock (1980a).

Although numerous studies on JH degradation have been published, most studies examine a single aspect of metabolism. Very few investigations have been complete enough to determine the relative importance of alternate pathways of metabolism or JH clearance. Most research has been in the Lepidoptera and no information is available on many orders of comparative interest. Hopefully, such information will soon be available.

Erley *et al.* (1975) extended the studies of Slade and Zibitt (1972) on the vagrant grasshopper, *Schistocera nitens* (syn *vaga*) and White (1972) on *Schistocera gregaria* to *Locusta migratoria*. JH I moved rapidly from an oil droplet into circulation and was rapidly excreted as unchanged JH, acid, diol, diol acid, and conjugates. Excretion rates were similar in adults of both sexes at various ages. This work has been continued at the biochemical level (Peter *et al.*, 1979b). Sams *et al.* (1978) evaluated JH metabolism in cultured fat bodies and ovary from the cockroach, *Periplaneta americana*. Other studies with the Orthoptera include those of Pratt (1975) and Hammock *et al.* (1977b).

Ajami and Riddiford (1973) found JH diol, acid, diol acid, and conjugates in the yellow mealworm, *Tenebrio molitor*. Subsequent workers have found high JH hydrolase as well as esterase at various times during development (Weirich and Wren, 1976; Mumby and Hammock, 1979a; McCaleb *et al.*, 1980; Reddy and Kumaran, 1980; Sparks and Hammock, 1980b). JH I is metabolized in the flour beetle, *Tribolium castaneum*, by ester hydrolysis, epoxide hydration, and apparent conjugation (Edwards and Rowlands, 1977). The Colorado potato beetle, *Leptinotarsa decemlineata*, has been the subject of many endocrine investigations and an impressive amount of information is accumulating on JH metabolism in this species (Kramer and deKort, 1976a, b; deKort *et al.*, 1978; Kramer, 1978). Metabolism is again dependent upon esterases, hydrolases and conjugating enzymes with little oxidative metabolism (Kramer *et al.*, 1977).

The Diptera often seem unlike other insects in their response to juvenoids, the amount of JH present and other factors. Their metabolism of JH also seems

somewhat unique. Slade and Zibitt (1972) found the conjugated diol ester to be a major metabolite in third instar larvae of the flesh fly, *Sarcophaga bullata*. Ajami and Riddiford (1973) failed to find JH diol in any of the four species of Diptera examined. They report evidence for oxidative metabolism although the very high R_f 'tetraol' metabolite probably indicates that it was actually a mixture consisting largely of tetrahydrofuran diols (Thf-diol, Figure 1.1). The diol acid does not appear as important a metabolite in the house fly, *Musca domestica*, as in many insects. JH diol and JH acid were found to be major metabolites in susceptible *M. domestica* while oxidative metabolism predominated in insecticide resistant strains (Hammock *et al.*, 1977a; Yu and Terriere, 1978a, b). Both cytosolic and microsomal JH esterases are present in homogenates of *M. domestica* larvae and these enzymes are quite unstable (Mumby *et al.*, 1979; Sparks and Hammock, 1980b). Following topical application to three stages of *Drosophila melanogaster*, Wilson and Gilbert (1978) found apparent conjugates, JH diol, and diol acid to be major metabolites with a trace of JH acid present. JH was rather stable in *D. melanogaster* haemolymph as earlier reported for haemolymph of *Sarcophaga bullata* (Weirich and Wren, 1976). Klages and Emmerich (1979a) also found no haemolymph JHE in larval *Drosophila hydei*, but esterase activity appeared in the pupal body fluid. Ester hydrolysis appeared to be the dominant metabolic pathway in tissue homogenates. Haemolymph JH esterase levels are very low in the honey bee, *Apis mellifera*, and metabolism seems to largely be due to tissue esterases (deKort *et al.*, 1977; Mane and Rembold, 1977). In two other species of Hymenoptera, Ajami and Riddiford (1973) also found the JH acid to predominate. Continued work on this order may help to answer interesting questions on the relationship of JH metabolism to social behaviour and host–parasite interaction as well as to facilitate more extensive comparisons of dipterous and hymenopterous insects.

Slade and Zibitt (1971, 1972) reported that the tobacco hornworm, *Manduca sexta*, hydrolyses the methyl ester of JH I and subsequently hydrates the epoxide. The diol ester is detected as an additional metabolite in *Hyalophora cecropia*. Ajami and Riddiford (1973) similarly found the diol to be a minor metabolite in pupae of each of three Saturniid species examined, but not in *M. sexta*. Conjugation pathways have not received adequate attention. Ajami and Riddiford (1973) and White (1972) present evidence for glucuronide and glucoside as well as sulphate formation. The most thorough study on insect conjugation of JH, to date, has been that of Slade and Wilkinson (1974) who report only sulphate formation in the southern armyworm, *Spodoptera eridania* (syn. *Prodenia*). Most subsequent work has been with *M. sexta*. These studies and similar work on several other lepidopterous species indicate that ester cleavage is the primary route of metabolism.

Information is lacking on the metabolism of JH in other arthropods. JH appears to have very low toxicity to mammals (Siddall and Slade, 1971; Slade and Zibitt, 1972), and it is rapidly metabolized in isolated rat hepatocytes

(Morello and Agosin, 1979). Very high levels of JH do interfere with macromolecular biosynthesis in cultured mouse cells (Chmurzyńska *et al.*, 1979 and bovine lymphocytes (Kensler and Mueller, 1978; Laskowska-Bożek and Zielińska, 1978; Zielińska *et al.*, 1978) as well as uncoupling oxidative phosphorylation (Chefurka, 1978). The high levels of hormone sometimes used suggest physical disruption of membranes.

Insect Epoxide Hydrolases

Epoxide hydrolases (E.C. 3.3.2.3, formerly E.C. 4.2.1.63 and known as hydrases or hydratases) add water to three-membered epoxide rings to yield 1,2-diols or glycols (Figure 1.1). Knowledge of insect epoxide hydrolases lags far behind knowledge of mammalian epoxide hydrolases (Oesch, 1973; Hammock *et al.*, 1980; Lu and Miwa, 1980). The high level of interest in mammalian epoxide hydrolases has stemmed from interest in the mutagenicity and carcinogenicity of some highly reactive epoxides such as the arene oxides and other natural and man-made xenobiotics. It is interesting, however, that the first demonstration of epoxide formation in mammals emerged from the study of an exceedingly non-reactive epoxide, the cyclodiene insecticide heptachlor (Davidow and Radomski, 1953). Initial work on insect epoxide hydrolases involved the investigation of the metabolism of these cyclodiene epoxides and only later involved the study of JH and juvenoid metabolism. In an early and very comprehensive study, Brooks *et al.* (1970) demonstrated that dieldrin and several related cyclodiene epoxides are metabolized very slowly by microsomal epoxide hydrolases in both insects and mammals. The relative importance of microsomal metabolism and epoxide hydration was nicely shown by comparing metabolism and toxicity of the dieldrin analogues HEOM and HCE (which are degraded largely by hydratio and hydroxylation, respectively) in several insects and vertebrates (Brooks *et al.*, 1970; El Zorgani *et al.*, 1970; Nelson and Matsumura, 1973; Walker and El Zorgani, 1974; Brooks, 1977, included references). The readily hydrated HEOM has been used as a model substrate for several subsequent studies of insect epoxide hydrolases (Brooks, 1973, 1974; Slade *et al.*, 1975, 1976; Craven *et al.*, 1976). Although no highly active and specific inhibitors were found in these studies, a wide variety of compounds including JH, juvenoids methylene dioxyphenyl, and benzothiozole mixed function oxidase (MFO) inhibitors, and organophosphates were found to cause some inhibition. Trichloropropene oxide and tetrahydronaphthalene 1,2-epoxide, which are inhibitors of styrene oxide epoxide hydrolase in mammals, also inhibited HEOM hydrolases in insects, although kinetics of inhibition were different. A very interesting observation was that some glycidyl ethers proved to be effective inhibitors of the insect enzymes. For a more comprehensive review of hydration of cyclodiene epoxides see Brooks (1977) and included references.

Compared with published work on insect esterases, investigation of the involvement of epoxide hydrolases in JH metabolism has been very limited. The tedious nature of the assays involved and the lack of effective hydrolase inhibitors have certainly limited the progress of research in this area. The most rapid assays involve either incomplete partitioning or the use of cellulose prelayer t.l.c. plates (Mumby and Hammock, 1979a; Mullin and Hammock, 1980). Although a continuous assay has been developed for epoxide hydrolases using *trans*-stilbene oxide, this substrate may be hydrated by enzymes not involved in JH metabolism. As illustrated by the early surveys of Slade and Zibitt (1971, 1972), White (1972), and Ajami and Riddiford (1973), the relative importance of ester hydrolysis and epoxide hydration in JH metabolism is variable in different insects, and the careful *in vivo* and *in vitro* kinetic studies to delineate the role of epoxide hydration in JH action are still lacking.

Using the juvenoid R-20458 (Figure 1.2), epoxide hydrolase activity was shown to be membrane bound and largely in the microsomal fraction of house fly, *M. domestica,* heads, abdomens, and thoraces, while little activity was detected in the soluble subcellular fraction. The hydration was fastest at neutral pH in *M. domestica* and distinct pH optima were observed, suggesting the involvement of several enzymes. A variety of compounds failed to cause strong inhibition of hydrolase activity, but the poor inhibition caused by trichloropropene oxide was especially notable (Hammock *et al.,* 1974a). Yu and Terriere (1978a, b) demonstrated that most hydrolase activity on JH I was microsomal in the house fly *M. domestica,* flesh fly *Sarcophaga bullata*, and black blow fly, *Phormia regina.* As reported for the metabolism of JH I and R-20458 for three strains of *M. domestica* (Hammock *et al.,* 1977a), it was reassuring that Yu and Terriere noted no major differences in hydrolase levels in insecticide susceptible and resistant strains. Possibly, epoxidized juvenoids may thus offer some advantages for insect control (Siddall, 1976; Zurflüh, 1976; Sparks and Hammock, 1980a). Yu and Terriere (1978b) report induction of JH I hydrolase activity by phenobarbital, and Mullin and Wilkinson (1980a) similarly observed induction in *Spodoptera eridania* midguts following exposure to pentamethylbenzene. When expressed in terms of metabolism per *M. domestica* equivalent, Yu and Terriere (1978a, b) observed high hydrolase activity towards the end of the last larval instar, a drop in the pupa, and a subsequent increase during the first two weeks of adult life. Several juvenoids inhibited JH I hydration, suggesting hydration by the same enzyme, while cyclodiene insecticides, several synergists, styrene oxide, and cyclohexene oxide caused no inhibition.

Slade and Wilkinson (1974) found JH epoxide hydrolase activity absent from the haemolymph, but widely distributed in other tissues from *Spodoptera eridania, H. cecropia, M. sexta,* and the cockroach, *Gromphadorhina portentosa.* The activity was largely microsomal. Hydrolase activity was similarly reported from the cockroach, *Periplaneta americana; T. molitor;* cabbage looper, *Trichoplusia ni;* and *M. sexta* (Hammock *et al.,* 1974a). Yawetz and Agosin

(1979) report epoxide hydrolase in epimastigotes of *Trypanosoma crazi* and speculate that it may be involved in degrading the hosts' JH. Slade *et al.* (1975 further demonstrated that HEOM hydrolase activity was largely membrane bound in *S. eridania* and *G. portentosa* midgut homogenates. The hydrolase pH optima for these two insects as well as for the blow fly, *Calliphora erythrocephala,* was quite basic. The lower pH optimum for JH hydration compared to HEOM and the weak inhibitory action of trichloropropene oxide on JH hydration suggest that different hydrolases may be involved with the metabolism of these two substrates. However, studies with the mammalian hydrolases have demonstrated that apparently the same enzyme can show greatly differing pH optima with different substrates and hydration of substrates with low apparent K_ms is poorly inhibited while substrates with high K_ms are effectively inhibited by trichloropropene oxide. Mumby and Hammock (1979a) found a relatively low apparent K_m (7.1 μM) for the hydration of R-20458 by *M. domestica* larval microsomes.

Slade *et al.* (1976) monitored epoxide hydrolase activity during the development of *S. eridania* using HEOM and JH I as substrates. As later verified on a separate study with the same species (Mullin and Wilkinson, 1980a), hydrolase levels were maximal in midgut homogenates during the middle of the last instar. Similar results were obtained by Wing *et al.* (1980) as they found the highest fat body hydrolase activity on R-20458 between the two major esterase peaks in the last larval instar (Figure 1.3).

Mullin and Wilkinson (1980a, b) reported the purification of a Cutsum^R-solubilized epoxide hydrolase from midgut microsomes of *S. eridania.* Five purification steps yielded the most active hydrolase reported from any eukaryote when styrene oxide or 1,2-epoxyoctane were used as substrates. The lack of activity on either HEOM or JH demonstrates that there are multiple forms of insect hydrolases and illustrates the need for further biochemical studies on these enzymes.

JH Esterases

JHE assay methods

Numerous methods have been developed for monitoring JHE activity since Weirich *et al.* (1973) first used a thin layer chromatography (t.l.c.) assay. Weirich and Wren (1973) extended this technique to a substrate specificity study in *M. sexta* haemolymph where they found that the esterase was specific for methyl esters and 2*E* geometry. Chromatographic assays using radiolabelled JH are certainly the most definitive methods since they can potentially discern other pathways of metabolism; however, they are laborious and the relative R_fs of JH and JH diol are variable depending upon the acidity of the chromatographic media and other factors (Hammock and Sparks, 1977). The use of cellulose

prelayer plates has greatly increased analytical speed in this laboratory; however, he reaction must be carefully terminated and chemiluminescence may create radio-counting problems. Higher resolution can be obtained through the use of high performance liquid chromatography (h.p.l.c.) (Morello and Agosin, 1979). Yu and Terriere (1975a) used a gas–liquid chromatographic assay for juvenoids, while Pratt (1975) used an electrophoretic assay. Sanburg *et al.* (1975a) report a rapid method using methoxy-labelled JH, and this method has been slightly modified for use in several laboratories (Nowock *et al.*, 1976; Hammock *et al.*, 1977b; Vince and Gilbert, 1977; Hwang-Hsu *et al.*, 1979; Klages and Emmerich, 1979a; Sparks *et al.*, 1979a). Unfortunately, the methoxy-labelled JHs are not commercially available. Hammock and Sparks (1977) report a rapid partition method using a commercially available JH I which can be modifed to monitor EH activity (Mumby and Hammock, 1979a). The method is also applicable to JH III analysis (Jones *et al.*, 1980; Wing *et al.*, 1980). Continuous assay for JHE and assays suitable for JHE staining on gels are still lacking. Analysis by pH stat and u.v. shift of the conjugated carbonyl proved too insensitive with JH and several model substrates. Coupling the hydrolysis of ethyl esters of JH and some analogues to alcohol dehydrogenase was only successful in the case of *M. domestica* because the JHE of many insects will not hydrolyse ethyl esters (Hammock *et al.*, 1977b, and unpublished work); however, such an assay may prove useful in some insects such as *T. molitor* and *Samia cynthia* (Weirich and Wren, 1976). The hydrolysis of β-methylumbelliferone esters was effectively used to monitor esterase activity during partial purification of JHE from *G. mellonella* haemolymph, but these compounds have not yet been shown to be specifically hydrolysed by JHE (Rudnicka *et al.*, 1979; Sláma and Jarolím, 1980). Several studies have demonstrated that α-naphthyl acetate (α-NA) is a poor marker for JHE activity in several insects (Weirich *et al.*, 1973; Hammock *et al.*, 1977b; parks and Hammock, 1979a); thus, one must be cautious about drawing conclusions regarding JH metabolism from model substrates.

JHE changes during development

The first developmental study of JH metabolism was that of Weirich *et al.* (1973) in *M. sexta*. Very little JHE activity was found in haemolymph from early instars or pupae of either sex. A major prewandering peak (Figure 1.3A) and a minor prepupation peak (C) of JHE activity were found which did not correlate with α-NA esterase levels. Subsequent studies in the Lepidoptera verified the report of Weirich *et al.* (1973). Numerous studies have dealt with monitoring α-NA hydrolysis during insect development. Since JH has often been shown to be hydrolysed by esterases different from those hydrolysing α-NA, the relevance of such work to JH metabolism is questionable. Brown *et al.* (1977) extended such an electrophoretic comparison of *Diatraea grandiosella* (southwestern corn

borer) esterases to define those esterase bands inhibited by JH and several juvenoids.

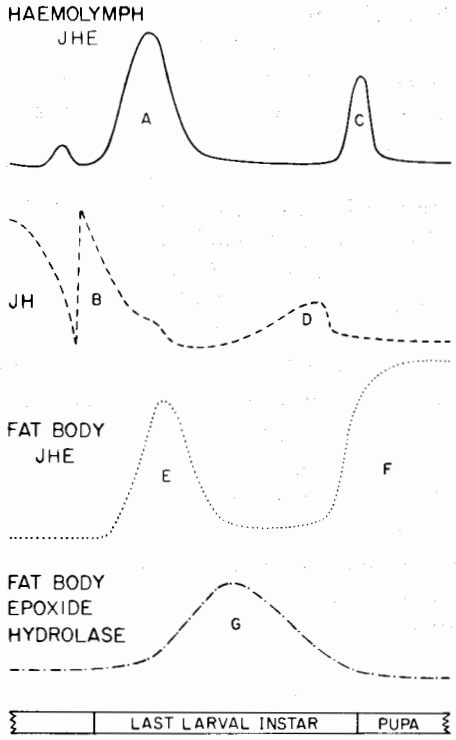


Figure 1.3 Relative levels of JH and enzymes possibly influencing JH titre during the last instar of a hypothetical lepidopterous larva. Juvenile hormone esterase (JHE) activity in the haemolymph (——) demonstrates a prewandering (A) and a prepupation (C) burst of activity. JH titre (– – –) drops at the moult, increases in the early last instar (B) and increases again before pupation (D). The increase in fat body JHE (· · · ·) in the prewandering last instar (E) coincides with the haemolymph increase (A), but the levels again increase in the prepupa (F) to remain high during most of the pupal stage. Epoxide hydrolase levels (– · – · –) are highest in the mid–last instar (G)

Sanburg *et al.* (1975a) monitored DFP sensitive and resistant esterase activity in fourth and early fifth instar *M. sexta* haemolymph. Vince and Gilbert (1977) reported occurrence of the major JHE peak just before *M. sexta* clears its gut and correlated the prepupation peak with the formation of tanned, sclerotized bands in the prepupa. Similar patterns of JHE activity were found in black mutant and allatectomized *M. sexta* except that the first JHE peak was smaller in both cases and the second JHE peak was greatly reduced in allatectomized larvae (Riddiford and Hammock, unpublished; Jones *et al.*, unpublished). Mitsui *et al.* (1979) found *M. sexta* epidermis degrades JH largely by ester hydrolysis and the half-life of JH in epidermal cultures is lowest when haemolymph JHE activity

peaks. Weirich and Wren (1976) report a large prewandering JHE peak in the saturniid *Samia cynthia* (Figure 1.3A), while Slade *et al.* (1976) monitored JH hydrolysing enzymes during the development of *Spodoptera eridania*.

Sparks *et al.* (1979a) studied hydrolysis of JH I and JH III in *T. ni* larvae. No difference was noted in male and female larvae and the second JHE peak was much larger than that reported in *M. sexta*. The second peak occurred early in the morning of the day of pupation regardless of what gate larvae were used (Sparks and Hammock, 1979b). Wing *et al.* (1980) monitored haemolymph and tissue JHE and EH as well as haemolymph JH binding during *T. ni* development. Peak EH levels lag behind JHE levels, and there is a correlation between fat body JHE and haemolymph JHE until pupation when the fat body JHE remains high (Figure 1.3). Hwang-Hsu *et al.* (1979) monitored JHE and α-NA esterase activity during development of *G. mellonella* as well as ecdysone titre and sensitivity to JH. The sharp peaks of JHE observed in *M. sexta* and *T. ni* were not reported, but there is JHE activity in the prewandering stage and activity remains reasonably high until pupation. In a survey of haemolymph JHE from several Lepidoptera, both the prewandering and prepupation JHE peaks generally occurred with JHE levels usually lower in butterfly larvae than moth larvae (Wing *et al.*, Jones *et al.*, unpublished). As indicated by Akamatsu *et al.* (1975), most workers who have monitored JHE have hypothesized an *in vivo* role for the enzyme based on correlations with expected decreases in JH titre. Indeed, in several insects the appearance of the first JHE peak correlates with the drop in haemolymph titre of JH, and haemolymph JH has again been detected in postwandering larvae prior to the prepupal JHE peak. Nijhout (1975), however, was quite justified in questioning the role of JHE in clearing JH from *M. sexta* haemolymph. By applying EPPAT (*O*-ethyl-*S*-phenyl phosphoramidothiolate) which inhibits JHE but not EH and other esterase inhibitors, Sparks and Hammock (1980b) demonstrated that inhibition of JHEs stabilized radioactive JH *in vivo*, and caused developmental aberrations consistent with stabilizing intrinsic JH. These studies provided direct evidence for the hypothesis that JHE actually play a role in normal JH metabolism *in vivo* in at least one insect.

Studies on JH metabolism in *L. decemlineata* have proved very useful because of the large background of biological and endocrinological literature on this beetle. Kramer and deKort (1976a) found low haemolymph JHE in third instar, but high JHE in fourth instar larvae which would correlate with a presumed decline in JH before pupation. High JHE was found in beetles just before diapause, again correlating with decreasing JH titres. Unlike the Lepidoptera examined, Kramer and deKort (1976a) report that developmental profiles of JHE and α-NA esterase were similar in *L. decemlineata* and speculate that JHE activity may comprise a substantial portion of the observed esterase activity on α-NA. Injections of Triton X-100 increased the half-life of radiolabelled JH in short-day beetles and inhibited JHE and EH. These studies indicate an *in vivo* role for JH metabolism in clearing at least exogenously administered JH (Kramer

et al., 1977). Using *T. molitor*, Weirich and Wren (1976) report very high levels of JHE in pupal and pharate adult haemolymph, lower levels in larvae, and very lo levels in adults. Reddy and Kumaran (1980) examined α-NA esterase and JH during the development of carefully timed *T. molitor* and found a rapid increase of JHE in prepupae, slowly decreasing levels of JHE during the pupal stage and low levels in the adult. Sparks and Hammock (unpublished) found very high levels of JHE in the 100,000*g* soluble fraction and high epoxide hydrolase levels in the 100,000*g* pellet of *T. molitor* pupal homogenates during development, but no difference in enzyme activity was noted between male and female insects. Edwards and Rowlands (1977) monitored *in vivo* JH I metabolism in the flour beetle, *Tribolium castaneum*, at eight times during development. Metabolism by both hydrolase and esterase pathways was highest in pupal and last larval instars and was lower in adults and early larvae.

JH metabolism in the Diptera seems to vary greatly from species to species and to be quite different from other insects. Yu and Terriere (1978a, b) followed esterase, oxidase, and hydrolase action on JH during the development of *M. domestica*. Activities were high during the larval stages, low in the early pupae, and increased prior to eclosion. These workers also compared the activities in three fly species and four strains of *M. domestica*. Differences were noted, especially in oxidase activity, which raised the question of how JH is regulated in insecticide resistant strains which rapidly metabolize it (Sparks and Hammock, 1980a). Wilson and Gilbert (1978) conclude that JH metabolism may not be a major mechanism of JH titre regulation in *D. melanogaster*. Klages and Emmerich (1979a) report that JHE in haemolymph and body fluid of *Drosophila hydei* larvae is very low, but that it increases in prepupae and pupae. Metabolism of JH is much higher in the fat body and body wall, due largely to a cytosolic esterase.

Mane and Rembold (1977) followed JH metabolism in the 20,000*g* supernatant of homogenates of queen and worker honey bee, *Apis mellifera*, larvae and pupae. As reported earlier for adults (Ajami and Riddiford, 1973), ester hydrolysis accounted for the majority of the metabolism. The activity was soluble at 100,000*g* and was highest in late larval instars and pupae. Interestingly, the activity was much higher in queen than in worker larvae.

The metabolism of JH at various times during development has been examined in several insects. With techniques now well established for monitoring *in vivo* metabolism of JH as well as measuring the major enzymes involved in its degradation, careful metabolism studies need to be extended to a variety of precisely timed insects from different orders.

JHE inhibition

The first indication that JH esterases in many insects were not 'typical' carboxylesterases stemmed from studies with inhibitors. Although α-NA

esterase activity which co-migrates with some JHE from *Hyalophora gloveri* pupae on electrophoresis can apparently be inhibited by *O,O*-di-isopropyl phosphorofluoridate (Whitmore *et al.*, 1972), JHEs are often much more resistant to inhibition than most serine esterases and proteases (Kramer *et al.*, 1974). Pratt (1975) in a screen of four compounds found paraoxon to be a good inhibitor of haemolymph JHE from the desert locust, *Schistocera gregaria,* and that inhibition was irreversible. Hammock *et al.* (1977b) demonstrated multiple forms of JH esterases in the cockroach *Blaberus giganteus* and screened 42 potential inhibitors using partially purified JHE fractions. Phosphoramidothiolates and *S*-phenylphosphates were found to be superb inhibitors; several classical esterase inhibitors were found to be very poor inhibitors, and most α-NA hydrolysis could be inhibited separately from JH hydrolysis. The studies with *S. gregaria* and *B. giganteus* also indicated that esterase inhibitors could be useful in biosynthesis studies by blocking subsequent JH metabolism (Hammock, 1975; Pratt, 1975; Hammock and Mumby, 1978). The use of such inhibitors can possibly be extended to studying *in vitro* action and receptor binding of JH as well as to *in vivo* kinetic studies.

Hooper (1976) reported a very extensive study of esterase action on malathion, α-NA, methoprene, hydroprene, and JH in *Culex pipiens pipiens*. He concluded that esterases hydrolysing malathion and α-NA are similar to each other and distinct from those hydrolysing JH. The enzymes could be classed as B- and A-esterases, respectively (Aldridge, 1953), and Hooper's (1976) studies illustrate the difficulty in forcing insect esterases into an artificial classification system designed for mammalian or avian esterases. JH esterases in the Diptera do appear to be quite different from the JH esterases in most other insects examined. In *M. domestica* larvae, activity was found in both the 100,000 ***g*** soluble and pellet (microsomal) subcellular fractions, and the activity in each fraction could be differentially inhibited. The soluble and especially the microsomal activity was quite unstable when compared to haemolymph or tissue JHE from Lepidoptera. *M. domestica* JHE activity was not inhibited by many of the compounds active on *T. ni, T. molitor,* or *B. giganteus,* but it was inhibited by a paraoxon and some *N*-ethyl carbamates (Yu and Terriere, 1978a; Mumby *et al.*, 1979; Sparks and Hammock, 1980b). These carbamates caused little or no inhibition of JHE from *B. giganteus, T. ni,* and *T. molitor*. The JHEs from *D. hydei* are also inhibited by several classical esterase inhibitors inactive on JHE from lepidopterous larvae, and Chang (unpublished) found that *N*-ethyl carbamates could greatly stabilize JH in *D. melanogaster* cell lines.

Kramer and deKort (1976b) report that haemolymph JHE of the Colorado potato beetle, *Leptinotarsa decemlineata,* are resistant to DFP but are very susceptible to inhibition by Triton X-100, and this compound has been shown to stabilize JH *in vivo* (Kramer *et al.*, 1977). The mechanism of Triton X-100 inhibition has not been elucidated, but there are several possibilities. One possibility is direct interaction with the enzyme, but another possibility is simply

sequestration of the JH substrate in micelles. JH not only forms micelles, but it is, undoubtedly, quite soluble in detergent micelles. The stability of JH in such micelles has been previously noted as a possible explanation for observations by Schmialek *et al.* (1975) on JH binding (Law, personal communication). It is known that some enzymes will not recognize substrate in micelle form (Mumby and Hammock, 1979b,c; Hammock *et al.*, 1980), and Armstrong *et al.* (1980) demonstrated that substrate sequestration is the mechanism by which non-ionic detergents inhibit the activity of a mammalian epoxide hydrolase. The affinity of such detergent micelles for lipophilic molecules may be quite high: even approaching the affinity of the *M. sexta* carrier protein for JH. Thus, detergent micelles may provide a nice model for the study of JH carrier–enzyme interaction, and the lack of Triton X-100 inhibition of JHE in the Lepidoptera may be of biological significance.

Triton X-100 also selectively inhibits haemolymph JHE in *T. molitor* while DFP and paraoxon are poor inhibitors (McCaleb *et al.*, 1980; Reddy and Kumaran, 1980); EPPAT emerged as a useful inhibitor of JH hydrolysis in soluble and microsomal fractions of *T. molitor* (Sparks and Hammock, 1980b). Mane and Rembold (1977) report that high levels of Triton X-100 inhibit *A. mellifera* JHE. Such differential inhibitors of JHE as Triton X-100, EPPAT, paraoxon, and DFP may be useful to investigate the apparent dichotomy of JHE in various insect groups as well as the physiological role of esterases. Ajami (1975) screened 16 potential esterase inhibitors using *M. sexta* pupal haemolymph. The haemolymph, if free of fat body, is rich in carboxylesterase, but JH hydrolysis is slow. Ajami (1975) was able to stabilize JH *in vivo* and demonstrate a synergistic effect of some esterase inhibitors. Slade and Wilkinson (1974, and unpublished) took an analogous approach and demonstrated *in vivo* stabilization of JH in the southern armyworm, *Spodoptera eridania,* using juvenoids and a range of EH and MFO inhibitors. In a comparison of 27 potential esterase inhibitors in three insects, Sparks and Hammock (1980b) report that EPPAT is a potent inhibitor in *T. ni* and *T. molitor,* but not in *M. domestica.* EPPAT will delay pupation by inhibiting the first JH esterase peak in the last larval instar of *T. ni* thus stabilizing JH and apparently delaying PTTH release. This study, like the Triton X-100 study in *L. decemlineata* (Kramer *et al.*, 1977), demonstrated an *in vivo* role for JHE as discussed earlier. The trifluoromethylketones represent a second novel series of inhibitors where the polarized ketone is hypothesized to mimic the tetrahedral transition state involved in ester cleavage. The inhibition caused by these compounds is reversible and quite specific for JHE in *T. ni, M. sexta,* and *G. mellonella* (Sparks and Hammock, 1980b, and unpublished).

JHE inhibitors have proved useful for testing the similarity of esterases from subcellular fractions, from different developmental stages, from different tissues, and acting on different substrates (Hooper, 1976; Hammock *et al.*, 1977b; Sparks and Hammock, 1979b; Jones *et al.*, 1980; Wing *et al.*, 1980). There was early hope that JHE inhibitors might act as synergists for JH or juvenoids (Solomon and

Metcalf, 1974; Ajami, 1975), but several factors make such action unlikely. The problems involved in the registration of a synergized formulation and the cost effectiveness of such a formulation are major economic considerations. JH will certainly not be used as an insecticide, and the most promising juvenoids either lack an ester function or possess an ester stable to most esterases so that the need for synergists is limited to isolated cases (Bigley and Vinson, 1979b). There is the possibility that inhibition of JHE may lead to disruption of insect development due to intrinsic JH, and since JHE of several insects appear to be so specific, there is hope that highly selective chemicals can be developed. Sparks and Hammock (1980a) conclude that such JHE inhibition by some classical organophosphates might have led to sublethal effects observed with insects in the field. However, they further conclude that, at least in *T. ni*, the inhibition of JHE does not appear a promising course for the development of control agents for several reasons:

(1) JHE appears to be present in large excess so that almost total inhibition is needed for an *in vivo* effect.
(2) JH production as well as metabolism appear to be precisely regulated.
(3) the inhibitors would only be effective during narrow periods of development; and
(4) the *in vivo* effects would include prelongation of a potentially destructive instar.

However, as tools for studying insect development, specific inhibitors are vital, and inhibitors such as the trifluoromethyl ketone moiety could be used for active site studies, purification via affinity chromatography, and possibly to aid in distribution of a JH mimic by binding with the JHE. For direct use in insect control, the disruption of JH metabolism by inhibition of enzyme production or precocious production of the enzyme is more promising.

JHE regulation

Since JHEs appear to be involved in JH regulation, understanding the regulation of these and other metabolic enzymes is a logical extension of our present knowledge. Retnakaran and Joly (1976) demonstrated that cautery of the A and B neurosecretory cells of *Locusta migratoria* reduces JHE activity. Hopefully, more research will soon emerge with the adult Hemimetabola. Kramer (1978) illustrated a complex response of *L. decemlineata* JHE to exogenous JH, which may possibly be correlated with corpus allatum activity (Schooneveld *et al.*, 1979). Treatment of diapausing beetles with JH I or several juvenoids caused a JHE increase, and this increase could be blocked by the use of puromycin or actinomycin D. This apparent induction was largely blocked by neck ligations suggesting that the JH effect on the fat body was indirect. A series of experiments using long- and short-day beetles with and without allatectomy

suggest that the fat body's history of exposure to JH may influence its ability to produce JHE. Reddy and Kumaran (1980) report that in *T. molitor* pupae JF and ETB have no effect on haemolymph JHE while precocene II slightly depresses JHE.

Terriere and Yu (1973) demonstrated that JH, juvenoids, and β-ecdysone stimulate MFO activity in *M. domestica*. Reddy and Krishnakumaran (1974) followed MFO levels during the development of *G. mellonella* and found that JH reduced MFO activity *in vivo* and *in vitro* but that it was increased in JH induced superlarvae. Although these results have not been clearly tied to hormone metabolism, JH can be degraded by MFO. Such changes in MFO activity during development or in response to hormones or even dietary inducers may have an effect on JH titres (Terriere, 1980; Wilkinson, 1980). Downer *et al.* (1975) found that the juvenoid methoprene could depress the activity of a non-specific esterase in *Aedes aegypti*.

JHE regulation in the Lepidoptera has received the greatest attention. Evidence that JH could directly 'induce' its own metabolism was first provided by Whitmore *et al.* (1972). In a very meticulous study using *H. gloveri* and *H. cecropia*, Whitmore *et al.* (1974) subsequently demonstrated that the apparent induction was dose dependent; prevented by puromycin, cycloheximide, and actinomycin D; that it could be duplicated with fat body *in vitro*; and that the esterases produced *in vitro* were immunochemically similar to the ones produced *in vivo*. Attempts to specifically label the enzyme(s) were not successful. These studies suffered from two problems. One problem was interpreting the role of 'inducible' esterases in pupae theoretically devoid of JH. The second problem stemmed from the logical but probably incorrect assumption that α-NA hydrolysis could be used as a marker for JHE activity. In spite of these limitations, later studies have generally supported the conclusions of Whitmore *et al.* (1972, 1974), and no subsequent studies using more biochemically defined JHE have been so thorough. Reddy *et al.* (1979) using *G. mellonella* and Wing *et al.* (1980) using *T. ni* also demonstrated that JH application to pupae resulted in increased haemolymph JHE. Since pupal fat body has high JHE, this effect could result only from release, but the data of Wing *et al.* (1980) suggest that some esterase production is also involved (it is very difficult to prove unequivocally true induction).

Data of Hammock *et al.* (1975b) indicate that *M. sexta* fat bodies and imaginal wing discs, held in short term culture, release JHE activity. Nowock *et al.* (1976) report that the apparent release of JHE activity by fat body *in vitro* correlates with peak haemolymph JHE activity (Figure 1.3E) and that α-NA esterase activity appears to be under separate control. Nowock and Gilbert (1976) further demonstrated in *M. sexta* the JHE activity of the 100,000 ***g*** supernatant of fat body homogenate paralleled that of the haemolymph for the first half of the instar. Inhibitor studies indicated that JHE release was an active process and not cell leakage. Similarly, in *G. mellonella* the release of JHE by fat body *in vitro*

could be correlated with the haemolymph JHE (Reddy *et al.*, 1979). Wing *et al.* 1980) monitored fat body, midgut, and haemolymph JHE levels during *T. ni* development. The fat body and haemolymph JHE levels correlated well during the last larval instar (Figure 1.3E), but haemolymph JHE was low and fat body JHE high during the pupal stage (F). Isoelectric focusing and inhibition data indicated that the fat body and haemolymph JHE activity appeared to be due to the same enzyme, but that much of the lower midgut activity was due to enzymes of different isoelectric points. Such data support but still do not conclusively prove the fat body to be the source of haemolymph JHE, and it indicates that production and release of JHE may be under different control mechanisms.

Studies from several laboratories serve to build a theory of JHE regulation in Lepidoptera. The data have been largely obtained from *Hyalophora* sp., *G. mellonella*, *M. sexta*, and *T. ni*, so the following generalizations should be treated as only a working hypothesis. There are two peaks of haemolymph JHE activity in *M. sexta* and *T. ni* and the first peak in prewandering larvae (A) (Figure 1.3) correlates with the decline of JH in the early instar (B). The second peak (C) follows a presumed second burst of JH (D). In *G. mellonella*, ligation of early larvae or starvation partially blocks the subsequent appearance of JHE (Reddy *et al.*, 1979). This partial block may indicate that tissues other than those in the head are involved in stimulating JHE production or that these factors are released prior to ligation and are either relatively stable or have a delayed effect. McCaleb and Kumaran (1978) and McCaleb *et al.* (1980) subsequently demonstrated that JHE production is closely coupled to the cues that initiate the larval–pupal transformation by studying JHE levels in injured, chilled, and/or starved insects. The effects of ligation or starvation in *T. ni* (Sparks and Hammock, 1979a, b) are more dramatic, totally blocking the appearance of the first JHE peak (A) or quickly reducing haemolymph JHE levels (F) to the trace activity seen in earlier instars. The moisture content of the food and several nutritive factors apparently play a role in maintaining high haemolymph JHE with dietary protein having a major role in *T. ni*. Starvation causes an immediate increase in haemolymph JH titre in *M. sexta* (Cymborowski *et al.*, 1979) so it is of possible physiological significance that starvation either prevents JHE appearance or later causes a precipitous decline in haemolymph JHE titres in last instar *M. sexta* just as it does in *T. ni* (Riddiford and Hammock, unpublished). High levels of JH, ETB, or precocene II will reduce haemolymph JHE levels in early last instar *G. mellonella* and will also reduce the release of JHE by fat body held in short term culture (Reddy *et al.*, 1979). In *T. ni* JH will not increase haemolymph JHE in the penultimate instar larvae, but it will increase the activity in the first JHE peak (A). This JH effect is either not directly on the fat body or it must be in concert with other head factors, since JH application will not increase haemolymph JHE in isolated abdomens. Implantation of the brain or sub-oesophageal ganglion stimulates JHE production in chilled or ligated *G.*

mellonella larvae with the brain giving the highest activity (McCaleb *et al.*, 1980). Similar effects have been obtained with *T. ni* (and apparently *M. sexta*: Vince and Gilbert, 1977), but the suboesophageal ganglion appears more active than the brain. Jones *et al.* (1980) further found that the ability of brain and suboesophageal ganglion homogenates to induce JHE in isolated abdomens as well as the fat body's responsiveness to the homogenates was correlated with haemolymph JHE titre in *T. ni*. Perhaps a peak of brain and suboesophageal ganglion activity in the penultimate larval instar serves to prime the unresponsive fat body and there is some evidence that a head factor may be involved in actively turning off JHE production. In *T. ni* implantation of the brain-suboesophageal ganglion complex was much more effective in restoring JHE activity than either tissue alone. The data from *G. mellonella*, *M. sexta*, and *T. ni* are thus consistent with a neurohormone from the brain and/or suboesophageal ganglion controlling the first JHE peak.

Control of the second JHE peak appears quite different from the first peak. Sparks and Hammock (1979a) reported that after the wandering stage, JH and juvenoids could induce JHE in a dose dependent fashion in both normal *T. ni* larvae and isolated abdomens. This induction could be inhibited by application of the antihormone ETB (Sparks *et al.*, 1979b). Wing *et al.* (1980) found that JHE in the fat body of postwandering *T. ni* also increases following juvenoid application, JH application also increases haemolymph JHE levels in *G. mellonella* larvae just before pupation (McCaleb *et al.*, 1980). Several lines of evidence suggest that the second JHE peak is due, at least in part, to a response to natural JH (Sparks and Hammock, 1979a). Injection of the MFO inhibitor *O*-bromophenoxymethyl imidazole which is known to block JH biosynthesis in corpora allata of *Blaberus giganteus* (Hammock and Mumby, 1978) will reduce the level of the natural JHE peak, but will not block the response to exogenous JH in *T. ni*. Since such imidazole compounds have a variety of actions, these data must be treated cautiously. Allatectomy of *M. sexta* larvae causes a large reduction in, if not elimination of, the second JHE peak, although allatectomized larvae readily respond to JH or juvenoid application by increased JHE (Riddiford and Hammock, unpublished). Preliminary data from *M. sexta* and rather conclusive data from *T. ni* indicate that the two JHE peaks are caused by the same enzyme (Sparks and Hammock, 1979a). If the regulatory mechanisms outlined above are valid, this could be one of the rare examples in biology where the production of the same protein is under two completely different regulatory mechanisms at different times during development (Sparks and Hammock, 1979a, b).

A teleological interpretation can be inflicted on the above data using *T. ni* as a model. If adequate food is available, the mature larva will halt or reduce JH biosynthesis and increased JHE will begin to clear JH from the haemolymph preparing the insect for PTTH release and subsequent pupation. If adequate food is not available for development, the insect must quickly respond in *M.*

sexta and *T. ni* by increasing JH production and reducing JHE, thus extending the feeding stage. It is clearly not advantageous for JH to lead directly to a large increase in JHE at this stage of development, and neurosecretory control of JHE facilitates a rapid response to environmental factors. Once JH has been cleared and PTTH released, it seems important to reduce the high JHE levels and, in fact, this process may be active. The JHE activity must be reduced for a second prepupal increase in JH, and this increase in JH seems important to prevent precocious adult development of some tissues (Kiguchi and Riddiford, 1978; Cymborowski and Stolarz, 1979). It is critical for normal development that the JH titre is again reduced before pupation, so that the ability of JH to stimulate JHE directly, possibly in concert with other factors, is clearly of survival value. The role of high fat body JHE in the pupa remains unclear, but Reddy *et al.* (1979) and Wing *et al.* (1980) hypothesize that it may ensure JH removal before adult development. Although direct inhibition of the enzymes involved in JH metabolism does not appear promising for insect control, the stimulation or inhibition of their production at inappropriate times could clearly disrupt development.

Influence of JH Carriers on Metabolism

Juvenile hormone is not only susceptible to metabolism in insect haemolymph, it is also quite lipophilic and should tend to partition out of an aqueous compartment. The mechanism by which JH is transported in insect haemolymph was a subject of speculation for many years. The answer came from the discovery of JH carrier proteins in the Leptidoptera (Whitmore and Gilbert, 1972; Kramer *et al.*, 1974) and the most detailed subsequent work has been with members of this order. When present in high concentration, JH will bind to high capacity, low affinity lipoproteins in insect haemolymph. Whitmore and Gilbert (1972, 1974) found that six different lipoproteins from the saturniids *H. cecropia, H. gloveri,* and *Antheraea polyphemus* will bind JH *in vitro*, but only two will bind JH *in vivo*. Although a high affinity binding protein is also present in *H. cecropia* (Gilbert and Hammock, unpublished), the tremendous capacity of the lipoprotein component overshadows its presence. In most other Lepidoptera examined, the low capacity, high affinity binding protein probably accounts for most JH binding (Goodman and Gilbert, 1979; Kramer *et al.*, 1974; Kramer and Childs, 1977), and this protein has been most extensively studied in *M. sexta*.

The *M. sexta* carrier protein has been biochemically characterized as having an acidic pI, a molecular weight of 28,000 daltons, and a K_a for JH I of $\sim 10^{-7}$ M with one binding site (Kramer *et al.*, 1974, 1976a, b; Akamatsu *et al.*, 1975; Goodman *et al.*, 1978a) and its major site of biosynthesis appears to be the fat body (Nowock *et al.*, 1975, 1976; Nowock and Gilbert, 1976). The carrier protein demonstrates a high specificity for JH I, and even juvenoids which are

quite active in *M. sexta* fail to have a high affinity for the carrier (Goodman *et al.*, 1976; Kramer *et al.*, 1976b). The carrier does not significantly bind JH metabolites, shows much higher affinity for JH I than JH III, and distinguishes the correct optical and geometrical isomer (Kramer *et al.*, 1974, 1976b; Goodman *et al.*, 1976, 1978b; Peterson *et al.*, 1977; Law, 1978; Gilbert *et al.*, 1978; Schooley *et al.*, 1978).

It is likely that the carrier protein influences JH stability and distribution in several ways. Sanburg *et al.* (1975a, b) demonstrated that esterases from *M. sexta* could be classified as general or JH specific enzymes based, in part, on the inability of general esterases to degrade JH bound to the carrier. Hammock *et al.* (1975b) similarly found that the carrier protein stabilized JH when added to *M. sexta* fat bodies in short term cultures. These two studies clearly indicate that the carrier protein reduces unwanted metabolism of JH (Gilbert *et al.*, 1976). It is not yet clear if JH 'specific esterases' actually metabolize bound JH or simply rely on a high affinity for the hormone and mass action. The carrier protein also tends to keep JH in solution. Although JH is readily water soluble at far above physiological concentrations, it tends to partition into lipophilic depots. Whether the depot is fat body or simply a lipid droplet, binding protein retards the uptake of and shifts the equilibrium away from lipophilic compartments and into aqueous compartments (Hammock *et al.*, 1975b; Nowock *et al.*, 1976; Mitsui *et al.*, 1979). Thus, the carrier protein helps to ensure an equal distribution of JH through the insect. It is likely that JH will behave as a surfactant and JH I has been shown to form micelles with a critical micelle concentration of about 10^{-5} M when determined by several independent methods (Kramer *et al.*, 1974, 1976b; Mayer and Burke, 1976; Hammock *et al.*, 1977b). Since such surfactants may accumulate near cell surfaces, the carrier may be important in preventing such surface excess of JH (Akamatsu *et al.*, 1975). Although the carrier seems to have a JH protective role for much of the insect's life, it also probably aids in the rapid clearing of JH from the insects' body by keeping it in circulation and accessible to JH 'specific esterases' rather than sequestered in depots refractory to metabolism (Gilbert *et al.*, 1978; Goodman and Gilbert, 1978; Sparks *et al.*, 1979a). There was some early indication that the carrier might additionally influence JH action, but subsequent work indicates that JH probably acts as a free molecule (Sanburg *et al.*, 1975b; Mitsui *et al.*, 1979). Kramer and Law (1980), in a nice comparison of JH biosynthesis and metabolism in *M. sexta* and *L. decemlineata*, suggest that the specific carrier molecule present in the Lepidoptera allows more precise regulation of JH and, thus, lower synthesis rates. Although such roles for the carrier protein appear obvious, and they have been demonstrated in numerous *in vitro* systems, *in vivo* demonstrations are still lacking.

The carrier protein titre fluctuates much as total haemolymph protein does in the penultimate larval instar of *M. sexta* (Goodman and Gilbert, 1978). Fluctuations during the last larval instar when changes in JH titre are most

dramatic are of high interest, but this study has been complicated by high JHE titres. This problem was solved in *T. ni* using EPPAT to inhibit JHE. *T. ni* JH binding is largely due to a single protein with a similar titre and affinity for JH I and III as the *M. sexta* protein (Hammock *et al.*, 1977a; Sparks and Hammock, 1979b). JH I binding activity in *T. ni* haemolymph fluctuates only slightly during the last larval instar, being highest in mid instar (Wing *et al.*, 1980). Hopefully, similar information will shortly be available for *M. sexta*.

Similar studies with JH carriers have been done in the Indian meal moth, *Plodia interpunctella*, and *T. ni*, with results analogous to those in *M. sexta* (Ferkovich *et al.*, 1975, 1976, 1977; Ferkovich and Rutter, 1976; Hammock *et al.*, 1977b; Sparks *et al.*, 1979a; Sparks and Hammock, 1979b; Wing *et al.*, 1980). Surveys of several species of Lepidoptera indicate that low molecular weight, high affinity JH haemolymph carriers are present (Kramer *et al.*, 1976a; Kramer and Childs, 1977; Wing *et al.*, unpublished).

In several insects, from a variety of orders other than the Lepidoptera, low molecular weight carrier proteins have not been detected. In these insects, high molecular weight lipoproteins with low affinity for JH probably assume the role as JH carrier (Trautmann, 1972; Emmerich and Hartmann, 1973; Emmerich, 1976; Kramer and deKort, 1976b, 1978; Bassi *et al.*, 1977; Hammock *et al.*, 1977b; deKort *et al.*, 1977). An interesting exception was the report by Hartmann (1978) of a very high affinity lipoprotein separated from a large amount of low affinity lipoproteins in the haemolymph of the grasshopper, *Gomphocerus rufus*. Peter *et al.* (1979b) report a complex situation in *Locusta migratoria* in which both a large capacity, low specificity diglyceride carrier lipoprotein and a high affinity carrier protein exist. The high affinity carrier binds the natural (10R) isomer of JH III preferentially over the unnatural (10S) isomer and JH I. Unbound hormone is then metabolized by haemolymph esterases. Klages and Emmerich (1979b) report low affinity JH binding to a large molecular weight protein isolated from the haemolymph of third instar larvae of *Drosophila hydei*. Interestingly, they also report a small high affinity protein which is quite unstable. This discovery suggests that re-examination of insect haemolymph from orders other than Lepidoptera is warranted.

A demonstration of JH binding does not necessarily imply a physiological role. For instance, JH will even bind to some mammalian proteins (Mayer and Burke, 1976). Kramer and deKort (1978) demonstrated that lipoprotein-bound JH was poorly protected from *L. decemlineata* esterases so these proteins may serve largely for transport. The indication that lipoproteins from the haemolymph of the cockroach, *Periplaneta americana*, protect JH from metabolism in short term organ culture may indicate another physiological role for such proteins (Sams *et al.*, 1978) although they probably also are involved in transport of other insect lipids (Chino and Gilbert, 1971; Gilbert, 1974; Whitmore and Gilbert, 1974). Research is now needed which will define the *in vivo* roles of binding proteins in the dynamics of JH action.

DODECADIENOATES

Methoprene

Insect metabolism

Hammock and Quistad (1976) have reviewed the metabolism of methoprene (Figure 1.2) in insects by comparing its degradation to that of other juvenoids. Weirich and Wren (1973) using *M. sexta* first demonstrated that haemolymph esterases were unable to effectively hydrolyse methoprene. The refractory character of the isopropyl ester function was substantiated in *M. domestica* (Yu and Terriere, 1975a, 1977b), *P. regina*, and *S. bullata* by the same workers (Terriere and Yu, 1977). Yu and Terriere have also amply documented the importance of microsomal oxidases in methoprene degradation *in vitro* by flies. Quistad *et al.* (1975d) studied the *in vivo* degradation of methoprene in *Culex*, *Aedes*, and *M. domestica* larvae. A number of parameters (including olefinic isomerization, larval age, penetration, and synergists) were examined in an attempt to rationalize susceptibility as related to metabolism. Solomon and Metcalf (1974) reported the metabolic fate of methoprene applied to *Oncopeltus fasciatus* (milkweed bug) and *T. molitor*. With the aid of synergists these workers demonstrated metabolic activation via *O*-demethylation to the hydroxy ester which had fourfold greater biological activity in *O. fasciatus*.

Hammock *et al.* (1977a) explored the possible metabolic basis for resistance to methoprene in *M. domestica*. Larvae resistant to methoprene and cross-resistant larvae (R-dimethoate) both metabolized methoprene faster than susceptible larvae. Methoprene also penetrated more slowly into larvae of the resistant strain. Esterases, epoxide hydrolases, and olefinic isomerization of the 2E bond were inconsequential to the development of house fly resistance. Mixed functio oxidase activity was considerably enhanced in resistant larvae which agrees with the work of Yu and Terriere (1977b) using another cross-resistant strain of *M. domestica* (R-diazinon).

Brown and Hooper (1979) compared the metabolism of methoprene in susceptible and methoprene-resistant larvae of *Culex pipiens*. Increased detoxication was an important factor in the high resistance developed after 30 generations of laboratory selection. Highly resistant larvae contained 11% less methoprene but more significantly, those larvae produced 40% more polar conjugates than susceptible larvae.

Bigley and Vinson (1979a, b) observed the degradation of methoprene by the imported fire ant (*Solenopsis invicta*). Adults and pupae metabolized methoprene primarily by *O*-demethylation to yield the hydroxy ester while larvae and pharate pupae produced mainly methoxy acid by esterase action. Although methoprene and its primary metabolites were all shown by bioassay to have juvenile hormone

activity against *Solenopsis*, the hydroxy ester was particularly effective and alleged to be an activated metabolite (see Solomon and Metcalf, 1974). Adults not only produce principally the activated hydroxy ester, but by meticulous waste management, trophallaxis, and intimate social contact they distribute the insect growth regulator and its metabolites throughout the colony thereby providing a potential reservoir of insecticidal compounds. Bigley and Vinson (1979b) also modulated the degradation of methoprene by concurrent application of synergists (PB and DEF) and they suggest that synergists may be useful in extending the useful lifetime of methoprene in *S. invicta* bait formulations.

Mammalian metabolism

The fate of methoprene has been studied in several rodents, namely rats (Tokiwa *et al.*, 1975; Hawkins *et al.*, 1977), mice (Cline *et al.*, 1974), and a guinea pig (Chamberlain *et al.*, 1975). These studies were designed largely to follow the balance and distribution of radiolabel from methoprene and/or its metabolites; hence, there was minimal structure elucidation of degradation products. It became readily evident from this rodent work that although most radiolabel was readily excreted (Table 1.1), significant tissue residues remained upon termination of the animals. There was an apparent dichotomy of facile metabolism (considerable $^{14}CO_2$ evolution) and refractory elimination (high tissue residues). Whole-body autoradiographs of rats (Hawkins *et al.*, 1977) showed a very general distribution of radioactivity with a particularly high concentration in the adrenal cortex. Although the exact identity of tissue metabolites was not

Table 1.1 Mammalian metabolism of methoprene—distribution of radiolabel

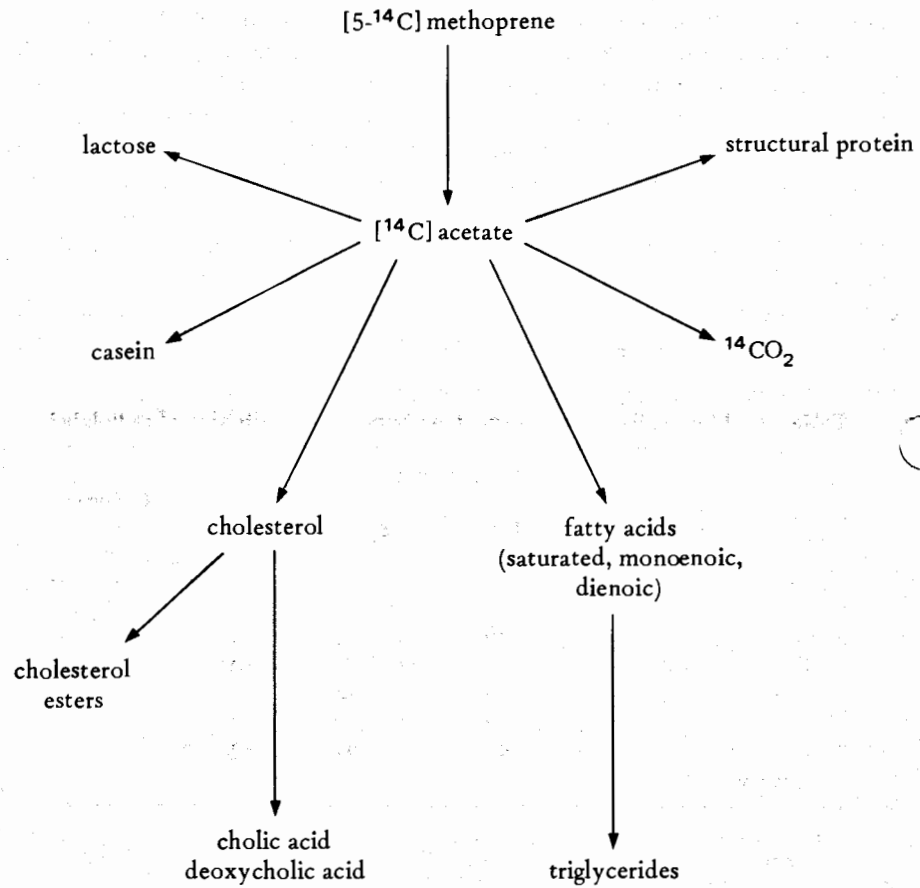
	Per cent applied dose					
	Steer (14 day)	Cow (7 day)	Rats (5 day)		Mice (4 day)	Guinea pig (1 day)
Urine	22	20	20[a]	18[b]	68	24
Faeces	39	30	18	35	14	9
$^{14}CO_2$	3	15	39	25	NA[c]	17
Tissues	13	20	17	13	0.1	NE[d]
Milk	—	8	—	—	—	—
Total recovery	77	93	94	91	82	50

[a] Hawkins *et al.* (1977).
[b] Tokiwa *et al.* (1975).
[c] [10-^{3}H]methoprene dosed at 0.9 mg kg^{-1}, Cline *et al.* (1974).
[d] Not examined.

pursued, in light of other concurrent work (Quistad *et al.*, 1974b, 1975b, c) the ^{14}C-residues were likely natural products (e.g. steroids) produced by extensive metabolism to anabolic precursors (e.g. [^{14}C]acetate).

The copious evolution of $^{14}CO_2$ from [5-^{14}C]methoprene was suggestive of extensive metabic degradation by mammals, particularly since the C-5 carbon was expected to be relatively inaccessible. A comprehensive investigation of the ^{14}C-tissue residues in bovines (Quistad *et al.*, 1974b, 1975b, c) confirmed that methoprene was exhaustively metabolized to common precursors of intermediary metabolism (e.g. acetate) which were then incorporated into natural products

Table 1.2 Bovine metabolism of [5-^{14}C]methoprene to natural products



(Table 1.2). The bovine metabolism of methoprene to primary metabolites in excrement (Chamberlain *et al.*, 1975) suggests partial degradation by the usual pathways of xenobiotic metabolism, but the quantitative abundance of acetate-derived ^{14}C-natural products and $^{14}CO_2$ implies that methoprene is also metabolized as a methyl-branched fatty acid (hence, as a 'food').

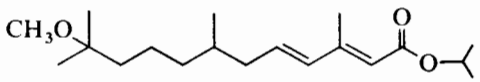
Environmental fate

Much of the environmental degradation of methoprene has already been reviewed (Quistad *et al.*, 1975e; Schooley and Quistad, 1979). As a conjugated dodecadienoate the ester is relatively stable to chemical hydrolysis at pH 5, 7, and 9 (Schooley *et al.*, 1975a). However, photochemical degradation is both rapid and extensive (Schaefer and Dupras, 1973; Quistad *et al.*, 1975a). An important practical photochemical reaction is the isomerization of 2*E*,4*E*-methoprene to the biologically inactive 2*Z*,4*E* isomer. In addition to the photoproducts given in Table 1.3, a plethora of products remains uncharacterized. Microbial degradation of methoprene in pond water (Schooley *et al.*, 1975a) and soil (Schooley *et al.*, 1975b) gave only primary metabolites rather than radiolabelled natural products which could be readily characterized. However, the facile biodegradability of methoprene was again evidenced by copious evolution of $^{14}CO_2$. Degradation of [5-^{14}C]methoprene by bluegill fish (Quistad *et al.*, 1976a) in an aquatic ecosystem gave a spectrum of radiolabelled natural products similar to that found in mammals (Table 1.2). Methoprene is also environmentally labile in the Metcalf ecosystem (Metcalf and Sanborn, 1975).

Methoprene is readily degraded by alfalfa and rice (Quistad *et al.*, 1974a). Although several primary metabolites were identified after enzymatic cleavage of conjugates, the majority of the 'metabolite' fraction consisted of a diverse array of products. Gel permeation chromatography strongly suggested incorporation of radiolabel from extensively degraded [5-^{14}C]methoprene into higher molecular weight plant constituents such as carotenoids and chlorophylls. The principal non-polar metabolite was 7-methoxycitronellal which was isolated from vapours transpired from plants (13% applied dose from rice).

Methoprene is considerably more stable to degradation under conditions necessary for stored products pest control. Rowlands (1976) found a residual half-life of 2–3 weeks for methoprene on freshly harvested wheat grain. Gel filtration chromatography revealed negligible inclusion of radiolabel into high molecular weight natural products (*vide supra*) and the methoxy acid represented 20–40% of the total degradation products. The stability of methoprene in stored tobacco is even more impressive. After 31 months of storage for [5-^{14}C]methoprene on Bright-leaf tobacco in a mini-hogshead ($6 \times 6 \times 60$ cm) to simulate natural storage conditions, 69% of the applied dose was recovered as intact methoprene (Staiger *et al.*, 1980).

Table 1.3 Environmental degradation products from methoprene



methoprene

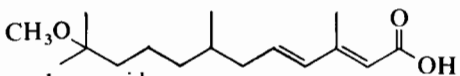
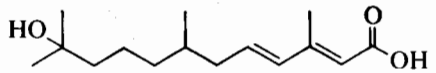
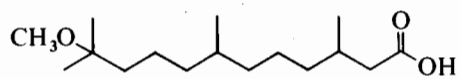
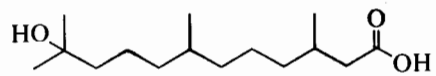
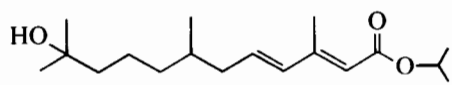
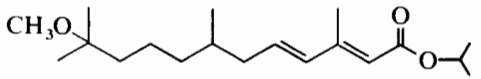
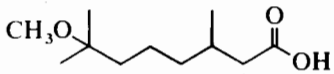
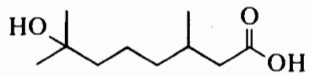
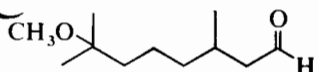
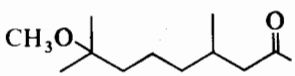
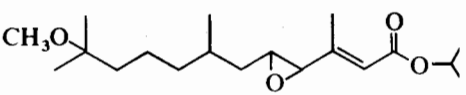
Degradation product	Produced by (maximum % applied dose, including conjugates)	Reference
 methoxy acid	Mosquito (7)	Quistad *et al.*, 1975d
	Meal worm (1)	Solomon and Metcalf, 1974
	Milkweed bug (0.4)	Solomon and Metcalf, 1974
	Fire ant (8)	Bigley and Vinson, 1979a
	Aquatic microbes (6)	Schooley *et al.*, 1975a
	Alfalfa (2)	Quistad *et al.*, 1974a
	Wheat grain	Rowlands, 1976
	Chicken (3)	Quistad *et al.*, 1976b
	Guinea pig	Chamberlain *et al.*, 1975
	Steer	Chamberlain *et al.*, 1975
 hydroxy acid	Mosquito (8)	Quistad *et al.*, 1975d
	House fly (10)	Quistad *et al.*, 1975d
	Fire ant (10)	Bigley and Vinson, 1979a
	Milkweed bug (0.3)	Solomon and Metcalf, 1974
	Mealworm (1)	Solomon and Metcalf, 1974
	Aquatic microbes (3)	Schooley *et al.*, 1975a
	Alfalfa (7)	Quistad *et al.*, 1974a
	Chicken (2)	Quistad *et al.*, 1976b
	Guinea pig	Chamberlain *et al.*, 1975
	Steer	Chamberlain *et al.*, 1975
 saturated methoxy acid	Chicken (6)	Quistad *et al.*, 1976b
 saturated hydroxy acid	Chicken (0.5)	Quistad *et al.*, 1976b
 hydroxy ester	Mosquito (9)	Quistad *et al.*, 1975d
	House fly (17)	Hammock *et al.*, 1977
	Fire ant (10)	Bigley and Vinson, 1979a
	Milkweed bug (0.4)	Solomon and Metcalf, 1974

Table 1.3 (*cont.*)



methoprene

Degradation product	Produced by (maximum % applied dose, including conjugates)	Reference
	Mealworm (2)	Solomon and Metcalf, 1974
	Aquatic microbes (7)	Schooley *et al.*, 1975a
	Soil (0.7)	Schooley *et al.*, 1975b
	Alfalfa (0.6)	Quistad *et al.*, 1974a
	Rice (0.9)	Quistad *et al.*, 1974a
	Blue gill fish	Quistad *et al.*, 1976a
	Guinea pig	Chamberlain *et al.*, 1975
	Steer	Chamberlain *et al.*, 1975
 methoxycitronellic acid	Mosquito (0.3)	Quistad *et al.*, 1975d
	House fly (0.3)	Quistad *et al.*, 1975d
	Aquatic microbes (29)	Schooley *et al.*, 1975a
	Alfalfa (0.8)	Quistad *et al.*, 1974a
	Rice (1)	Quistad *et al.*, 1974a
	Photochemical (7)	Quistad *et al.*, 1975a
 droxycitronellic acid	Mosquito (7)	Quistad *et al.*, 1975d
	Alfalfa (3)	Quistad *et al.*, 1974a
	Rice (0.3)	Quistad *et al.*, 1974a
 methoxycitronellal	House fly (0.6)	Quistad *et al.*, 1975d
	Alfalfa (2)	Quistad *et al.*, 1974a
	Rice (13)	Quistad *et al.*, 1974a
	Photochemical (13)	Quistad *et al.*, 1975a
	Photochemical (4)	Quistad *et al.*, 1975a
	House fly (3)	Hammock *et al.*, 1977
	Photochemical (6)	Quistad *et al.*, 1975a

Chickens metabolized methoprene by pathways similar to those in mammals (Davison, 1976; Quistad *et al.*, 1976b). Radiolabelled triglycerides and cholesterol in egg yolks, fat, and liver were again reflective of exhaustive degradation to [^{14}C]acetate followed by anabolism to natural products. A unique pathway in chickens involved *reductive* metabolism to hydrogenated hydroxy and methoxy acids. These reduced primary metabolites were important constituents of triglycerides (egg yolk, fat, liver) and the reduced methoxy acid was even esterified with cholesterol (liver). Metabolic saturation of the dienoate was dose-dependent, decreasing with a reduction in dose level.

Hydroprene

Insect metabolism

Terrier and Yu have studied in detail the *in vitro* metabolism of hydroprene by esterases and microsomal oxidases of house flies (Terriere and Yu, 1973; Yu and Terriere, 1975a) and also of blow flies and flesh flies (Terriere and Yu, 1977). Both esterolytic and hydrolytic cleavage were important pathways for hydroprene degradation by flies. Since the hydroprene molecule exhibits an absence of multiple functionality, the 2,4-dienoate chromophore is implicated as a likely site of microsomal oxidation. Indeed, subsequent work by Yu and Terriere (1977a) using the same dipterans revealed several metabolites resulting from epoxidation of double bonds in [5-^{14}C]hydroprene (Table 1.4). Several additional epoxidized products (Table 1.4) were characterized by mass spectral fragmentation patterns of metabolites from hydroprene in the red cotton bug (*Dysdercus koenigii*) (Tungikar *et al.*, 1978).

Environmental fate

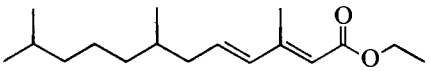
Since hydroprene is not as commercially developed as methoprene, it is not surprising that there is a paucity of detail concerning environmental degradation. Henrick *et al.* (1975) identified a 3-hydroxy-2-pyrone (Table 1.4) from the photosensitized oxygenation of hydroprene in ethanol, but the abundance of this photoproduct under environmental conditions is unexplored.

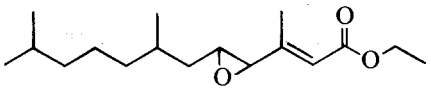
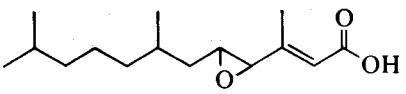
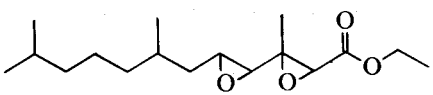
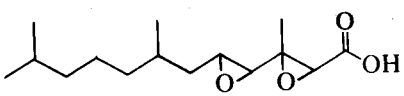
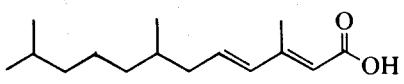
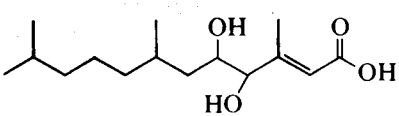
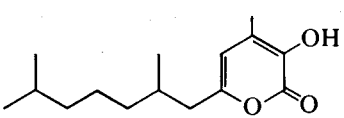
TERPENOID PHENOXY ETHERS AND RELATED COMPOUNDS

Insect Metabolism

The terpenoid phenoxy ether juvenoids will be treated together because the metabolic pathways involved appear common to most of the structures and the majority of the published research has dealt with a single compound, 1-(4-ethylphenoxy)-6,7-epoxy-3,7-dimethyl-2-octene (Figure 1.2) (R-20458 of

Table 1.4 Degradation products from hydroprene



Degradation product	hydroprene	Produced by	Reference
		House fly, flesh fly, blow fly	Yu and Terriere, 1977a
		Red cotton bug	Tungikar *et al.*, 1978
		Flies	Yu and Terriere, 1977a
		Red cotton bug	Tungikar *et al.*, 1978
		Red cotton bug	Tungikar *et al.*, 1978
		Red cotton bug	Tungikar *et al.*, 1978
		Flies	Yu and Terriere, 1977a
		Red cotton bug	Tungikar *et al.*, 1978
		Flies	Yu and Terriere, 1977a
		Photochemical	Henrick *et al.*, 1975

Stauffer Chemical Company). The first report on its metabolism in insects was a cursory study which showed the corresponding diol (K, Figure 1.4) as the only metabolite identified from the faeces of the American locust, *Schistocerca americana* (Gill *et al.*, 1972). R-20458 (0.1–1 μg/insect) will cause normal adult coloration, mating, and reproduction in allatectomized male and female *S. americana c.* 2 weeks after a single injection. This juvenile hormone-like effect is apparently similar to the juvenile hormone-dependent maturation in male and female *S. gregaria* (Loher, 1960). At the effective doses of either 0.25 or 1 μg/insect of R-20458, >90% of the radioactivity is excreted into the faeces with much of the remaining dose localized at the injection site (Loher and Hammock, unpublished; Hammock, 1973).

Subsequent studies with the juvenoid R-20458 indicated that insects could be divided into two groups based on the relative contribution of hydrolases and oxidases to the metabolism. For instance, the diol is a major metabolite in larvae and pupae of the yellow meal worm, *T. molitor,* while numerous metabolites are detected in the faeces of the cockroach, *P. americana* (Hammock *et al.*, 1974a). Similar metabolite distributions are found in tissue homogenates of a variety of insects including *T. molitor, P. americana, M. domestica, S. calcitrans, S. bullata, M. sexta,* and *T. ni* (Singh, 1973; Hammock *et al.*, 1974a, 1975a). Since the majority of reported research has been with the house fly, *M. domestica,* it will be used as an example. Ether cleavage does not appear important in insects, with the possible exception of *P. americana.* The major routes of metabolism involve hydroxylation at the alpha (benzylic) and beta positions of the ethylphenoxy moiety and, in some cases, oxidation to the corresponding ketone (Figure 1.5). In a strain of house flies which had been previously selected with Baygon and which was known to have high MFO activity, hydroxylation at the alpha position on the ethyl side chain was greatly enhanced. Of much less importance was epoxidation at the 2,3-position. In all insects examined *in vivo* or *in vitro,* epoxide hydration was an important route of metabolism as discussed in more detail above.

An interesting series of metabolites may be formed from the diepoxide (Figure 1.4). Since treatment of the epoxide of JH or R-20458 (J) with aqueous acid gives a diol (K), one might expect a tetraol (H) (Figure 1.4) to result following similar treatment of a diepoxide (F) (Gill *et al.*, 1972; Ajami and Riddiford, 1973). However, the 6,7-epoxide is much more labile to acid and, apparently more labile to enzyme catalysed hydration than the 2,3-epoxide (Mumby and Hammock, 1979b, c), so a diol-epoxide (G) results which cyclizes to a variety of 5-, 6-, and 7-membered heterocyclics, the most common of which are tetrahydrofuran diols (C,D) (Figure 1.4). Evidence for this cyclization was initially based on literature comparisons, chemical identification, synthesis of the tetrahydrofuran diols by alternative pathways, and n.m.r. detection of the ephemeral 6,7-diol-2,3-epoxide (G) following reaction of the corresponding olefin (K) with peracid (Hammock, 1973; Hammock *et al.*, 1974b). More recently, the 6,7-diol-2,3-epoxide was

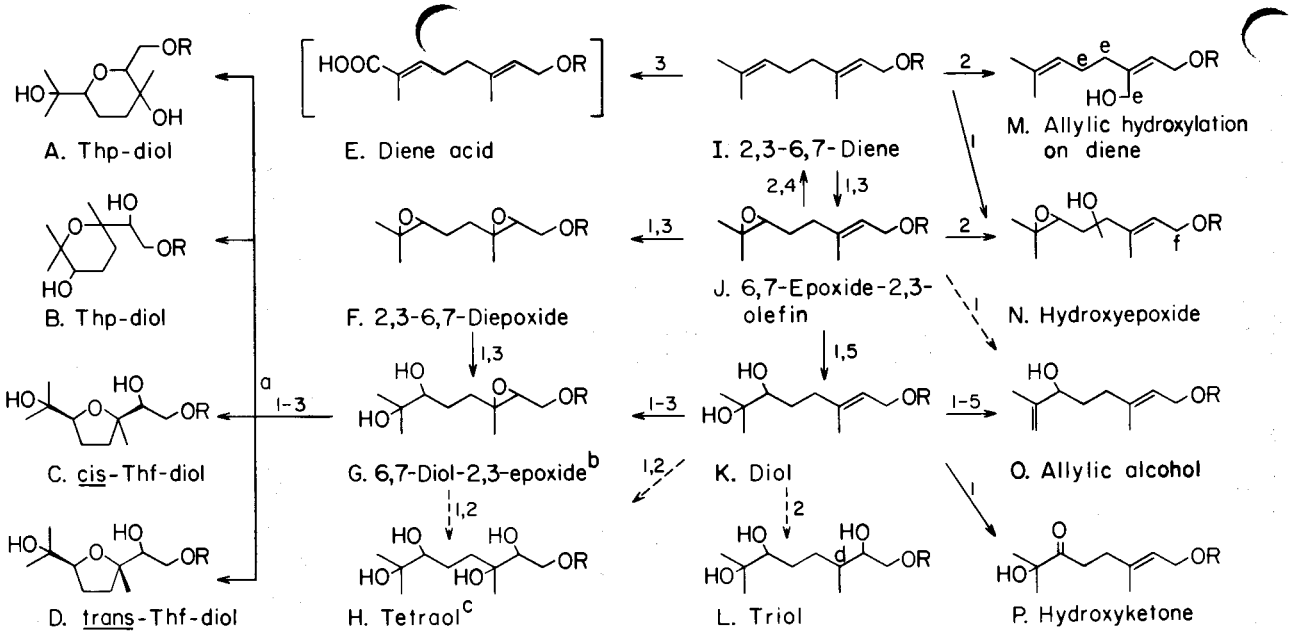


Figure 1.4 Pathways of metabolism and environmental degradation of the epoxy geranyl moiety of the juvenoid R-20458. Reference 5 concerns the related compound Ro-10-3108 which lacks the 2,3-olefin and has ethyl branches at C-3 and C-7. R = H or a phenoxy moiety (Figure 1.5). Numbers refer to literature references for the pathways: 1, Gill *et al.*, 1972, 1974: 2, Hoffman *et al.*, 1973; 3, Hammock *et al.*, 1974a, 1975a, 1977a; 4, Ivie, 1976; Ivie *et al.*, 1976; 5, Dorn *et al.*, 1976; Hangartner *et al.*, 1976. [a]The diol-epoxide rearranges to a variety of products including oxepane diols and bicyclic ethers, only the major products are shown (Hammock, 1973). [b]This unstable intermediate described in reference 2 had a m.s. appropriate for compounds C and D. [c]The tetraol does not form from either oxidation of the diol or hydration of the diepoxide *in vitro*, see text. [d]Hydroxylation also occurs at C-3. [e]Allylic hydroxylation occurs at several positions, and allylic hydroxylation followed by two dehydrogenation steps produces metabolite E. [f]Allylic hydroxylation alpha to the ether results in cleavage of the aryl alkyl ether

isolated following peracid oxidation of the corresponding olefin in a modified biphasic system. The diol-epoxide was chemically characterized, and its reactivity as an alkylating agent with 4-(*p*-nitrobenzyl)pyridine and its mutagenicity in the Ames' *Salmonella* assay determined. The compound was found to be quite reactive, but most reactions involved internal rearrangement rather than alkylation. For this reason, and possibly also for steric reasons, the diol-epoxide showed no detectable mutagenic activity (Ota and Hammock, unpublished). The proportions of furan, pyran and oxepane diols obtained following incubation of the diepoxide with acid, rat microsomes or house fly microsomes vary slightly, but isotope dilution techniques using authentic tetraol (H) demonstrated that less than 0.1% of the total hydration can be accounted for by these products (Hammock, 1973; Hammock *et al.*, 1974a, b; Gill *et al.*, 1974). Essentially, only tetrahydrofuran diols result following epoxidation of the 2,3-olefin of R-20458 diol (or the 6,7-olefin of JH diol) (Gill *et al.*, 1974; Hammock *et al.*, 1974a, b). However, the tetraol, as well as thf diols, clearly appears to be an *in vivo* metabolite in mammals as discussed below (Gill *et al.*, 1974).

As an approach to avoid the use of epoxidized juvenoids which are potentially unstable in the environment, one could use the corresponding olefins and rely on the insect to activate them to the epoxide *in vivo*. In a comparison of the metabolism of R-20458 (J) with its corresponding diene (I) and diol (K), the diene was metabolized at the highest rate to an olefinic acid probably by hydroxylation and subsequent oxidation of a C-7 methyl since hydroxy and aldehyde intermediates were formed. Although a small amount of the biologically active 6,7-epoxide was formed, the instability of the allylic methyl to microsomal oxidases limits the potential of such an approach in insect control (Hammock *et al.*, 1974b, 1975a).

The potential application of knowledge of relative activities of epoxide hydrolases and microsomal oxidases in test insects for design of juvenoids was illustrated by a comparison of the half-lives (in hours) of R-20458 and its 7-methoxy and 7-ethoxy analogues (Figure 1.6). In susceptible house flies with moderate hydrolase and oxidase activities, the respective half-lives were 0.5, 1.0, and 1.5 h while in resistant house flies with high MFO levels the half-lives were 0.3, 0.1, and 0.3 h with hydroxylation on the ethyl side chain rather than epoxide hydration accounting for most of the R-20458 metabolism. In stable flies with low hydrolase and moderate oxidase levels, the half-lives are 4.5, 3.5, and 6.5 while in *T. molitor* pupae with low oxidase activity the half-lives are 8.0, 57, 63 h (Hammock *et al.*, 1975a). The relative biological activities of epoxides and methoxides (ethoxides appear quite selective) in various insects largely reflect relative hydrolase and oxidase levels. Thus, for maximum activity on many field populations of resistant insects, juvenoids should be chosen which are refractory to attack by microsomal oxidases (Sparks and Hammock, 1980a). Studies on the metabolism of the geranyl phenyl ether alkoxides may facilitate interpretation

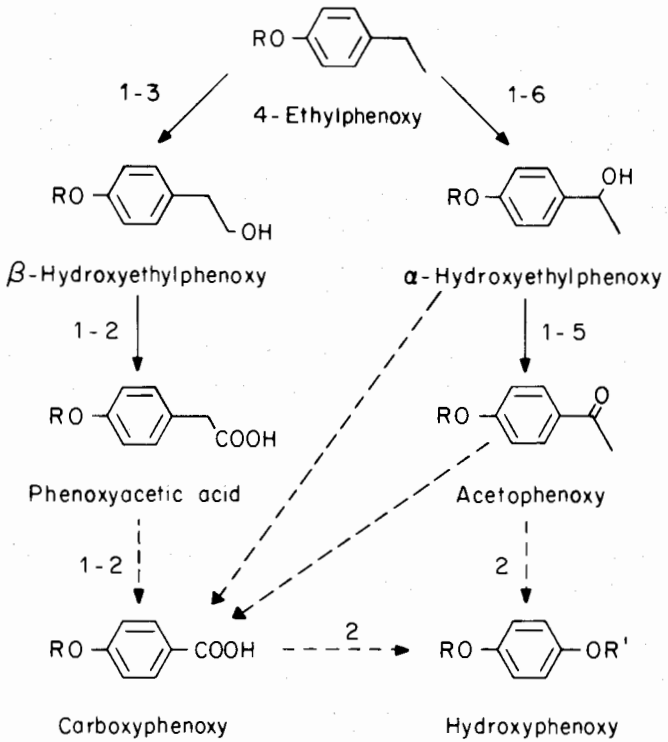


Figure 1.5 Pathways of metabolism and environmental degradation of the ethylphenoxy moiety of the juvenoids R-20458 and Ro-10-3108. R = a terpenoid moiety; R′ = H, SO_3H, or glucuronide. Solid arrows indicate established pathways and dashed arrows indicate possible routes leading to metabolite formation. Numbers refer to literature references for the pathways: 1, Gill *et al.*, 1972, 1974; 2, Hoffman *et al.*, 1973; 3, Hammock *et al.*, 1974a, 1975a; 4, Ivie *et al.*, 1976; 5, Dorn *et al.*, 1976; Hangartner *et al.*, 1976; 6, Agosin *et al.*, 1979

of stability data on various arylterpenoid derivatives (Schwarz *et al.*, 1974) (Figure 1.2).

Most of the metabolites of R-20458 and the 7-methoxy and ethoxy analogues were devoid of morphorgenetic activity in the *T. molitor* pupal bioassay (Hammock *et al.*, 1974a, b, 1975a). The 7-hydroxy (*O*-dealkylation) products (Figure 1.6) were active in some insects, and benzylic oxidation of R-20458 actually resulted in an active juvenoid reported earlier by Bowers (1969). Possibly derivatives of acetophenoxy or α-hydroxyethylphenoxy juvenoids could be used for insect control (Sláma *et al.*, 1978).

Wright and Spates (1975) investigated the penetration and persistence of R-20458 in the stable fly, *S. calcitrans*, while Hammock *et al.* (1977a) compared the

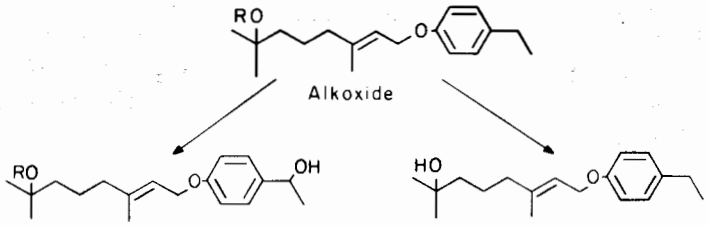


Figure 1.6 Major routes of metabolism of a 7 alkoxy geranylphenyl ether. R = CH_3 or C_2H_5

in vitro metabolism of methoprene, JH I and R-20458 in susceptible and methoprene resistant house flies. The diol was the only metabolite in the absence of NADPH, but a variety of metabolites resulting from oxidation of the ethylphenoxy and 2,3-olefin were detected with NADPH. As reported earlier with Baygon-resistant insects, metabolism was much faster in the methoprene-resistant strain. Since juvenoids represent a new structural approach to insect control agents, the appearance of site of action resistance will probably be delayed. However, one can safely predict cross-resistance in insect populations which have been heavily selected with classical pesticides. Sparks and Hammock (1980a) reviewed the resistance problem as related to insect growth regulators and discussed approaches to delay or circumvent resistance development.

Mammalian Metabolism

Several workers have examined the *in vivo* metabolism of R-20458. Following intraperitoneal administration to rats, Gill *et al.* (1972) reported 100, 96, and 73% recovery of radioactivity in excreta using the ^{14}C-phenyl-, ^{3}H-phenyl-, and geranyl labels respectively. The lower recoveries with ^{3}H-phenyl and geranyl labels were probably due to exchange and metabolic release, respectively. Hoffman *et al.* (1973) report quantitative recovery of radiolabel from rats following either oral or intraperitoneal administration of the ^{14}C-phenyl compound. Approximately equal amounts of radioactivity were recovered in the urine and faeces, and no radioactivity was detected as expired $^{14}CO_2$ or as tissue residue. Either 1 mg or 100 mg kg^{-1} doses of ^{14}C-phenyl R-20458 delivered orally to mice or intraperitoneally to rats resulted in almost quantitative recovery of the radioactivity in the urine and faeces within 96 h (Singh, 1973; Gill *et al.*, 1974). Following oral administration to rats at 1 mg kg^{-1}, maximum blood and liver levels of 11 and 26 p.p.m. relative to dry tissue weight, respectively, were reached at 0.5 h, and levels of ^{14}C decreased rapidly thereafter (Singh, 1973). Following oral administration of R-20458 to a steer, Ivie *et al.* (1976) reported >98% recovery with >84% of the dose in the urine and >14% in the faeces. Only trace (low p.p.b. or p.p.t.) levels remained in the tissues 7 days after oral administration. Seven days after dermal application of R-20458 to a steer, approximately

30% of the material had been absorbed and was rapidly eliminated in the urine and faeces.

As would be expected from a compound with so many sites labile to metabolism, a plethora of metabolites was observed in all cases. Much of the metabolism can, however, be explained on the basis of products resulting from a combination of several primary steps in metabolism. For simplicity, the metabolism of the phenoxy and geranyl portions of the molecule will be treated separately. As with insects, the major route of oxidative metabolism involves hydroxylation in the benzylic position of the ethylphenoxy moiety resulting in the α-hydroxyethylphenoxy moiety which can be dehydrogenated to acetophenoxy compounds (Figure 1.5). The less abundant β-hydroxyethylphenoxy compounds are presumably oxidized to phenoxyacetic acid derivatives and possibly decarboxylated to the carboxyphenoxy derivatives. Hoffman *et al.* (1973) reported the *p*-hydroxy compounds as rat metabolites and speculated that they might arise from a Baeyer–Villiger-like oxidation of the acetophenoxy moiety. Aromatic hydroxylation has not been confirmed, but one could safely predict that such pathways play, at least, a limited role in metabolism further increasing the number of possible metabolites. These phenoxy derivatives are present with the geranyl phenoxy ether intact with various modifications on the geranyl portions (Figure 1.4), as free phenols, and as several conjugates. Phenols and conjugates of phenols constitute a major portion of the metabolites from all mammals examined; for instance, in steers ~37% of the total radiocarbon existed as conjugates of either ethylphenol or acetophenol (Ivie *et al.*, 1976). Hoffman *et al.* (1973) speculate that formation of the acetophenoxy derivative facilitates subsequent cleavage of the geranyl phenoxy ether linkage.

Since an adequate label was lacking, metabolism of the epoxygeranyl portion of the R-20458 molecule was only followed while the ether linkage was intact. One might expect ether cleavage to occur by hydroxylation alpha to the allylic ether (N) (Figure 1.4). The resulting product would be expected to rearrange to the corresponding geranyl derivative and be subsequently dehydrogenated to the acid. Once a free geranyl derivative is formed, one would predict rapid metabolism to acetate and other natural products as shown for geraniol in *Pseudomonas citronellois* by Seubert and Fass (1964) and possibly incorporation into natural products as described for methoprene by Quistad *et al.* (1974b). A major discrepancy between *in vivo* and *in vitro* data is that ether cleavage is a major *in vivo* metabolic route which has not been detected in tissue homogenates or reconstituted P450 (Gill *et al.*, 1972, 1974; Agosin *et al.*, 1979). Possibly, such ether cleavage is catalysed by gut microorganisms.

The *in vivo* metabolism of the geranyl portion of the molecule involves epoxide hydration, epoxidation of the 2,3-olefin (possibly with subsequent cyclization), allylic hydroxylation, and possibly either reduction of the 2,3-olefin followed by hydroxylation or some form of reductive hydroxylation (Figure 1.4) (Gill *et al.*, 1972, 1974; Hoffman *et al.*, 1973; Ivie *et al.*, 1976). A search for metabolites of the

epoxide other than the corresponding diol was not fruitful since several possible rearrangement products were not detected (Gill *et al.*, 1974; Hammock *et al.* 1974a). Hoffman *et al.* (1973) reported fairly large amounts of the corresponding 6,7-olefin (I) (Figure 1.4) in rat faeces following administration of R-20458 which they attributed to olefinic impurities in material administered to the rat. Ivie *et al.* (1976) also found the 6,7-olefin as a metabolite from the steer, but traced it to an epoxide reduction occurring in the rumen fluid (Ivie, 1976). Subsequent work has shown that the contents of the large intestine of a variety of mammals, including man, when incubated under carbon dioxide will all reduce epoxides very cleanly, to the corresponding olefin (Hammock *et al.*, 1980b).

Hoffman *et al.* (1973) report metabolites resulting from hydroxylation of the terpenoid chain of R-20458 (N) (Figure 1.4) and the corresponding diene (M). Apparently, the 2,3-olefin can be reduced and hydroxylated resulting ultimately in a pair of triols (L). Epoxidation of the 2,3-olefin of either the epoxide or the diol occurs (F,G) but biological and chemical epoxidation of the 2,3-olefin is much slower than the 6,7-olefin (Gill *et al.*, 1972, 1974; Hammock *et al.*, 1974a, b, 1975a). The 6,7-epoxide is similarly much more labile to acid catalysed and enzymatic hydration than the 2,3-epoxide probably resulting in the ephemeral diol-epoxide intermediate (G) (Mumby and Hammock, 1979b, c). The diol-epoxide rapidly rearranges to a variety of cyclic and bicyclic products unless held under basic conditions (Ota and Hammock, unpublished). The most abundant of these products are shown in Figure 1.4 (A–D), but oxepane diols and bicyclic ethers also result from the rearrangement (Hammock, 1973; Gill *et al.*, 1974; Hammock *et al.*, 1974a, b). The mass spectrum of the diol-epoxide (G) reported as a metabolite in rats is consistent with the mass spectra of the tetrahydrofuran diols (C and D). The tetraol (H) clearly does not arise from hydration of either the diepoxide or the diol-epoxide *in vitro*, but the support for the *in vivo* formation of the acetophenoxytetraol is quite good (Hoffman *et al.*, 1973; Gill *et al.*, 1974). Possibly a very rapid oxidation–hydration occurs before cyclization in some tissues or in the gut. The acetophenoxy moiety might also encourage hydration over cyclization.

Although many metabolites are present, most of the dose of R-20458 given to mammals can be accounted for by hydroxylation at the benzylic position of the ethylphenoxy moiety, epoxide hydration, and ether cleavage. Subsequent conjugation of the resulting phenols, presumably as sulphates and glucuronides, appears to be extensive (Hoffman *et al.*, 1973; Gill *et al.*, 1974; Ivie *et al.*, 1976).

With the exception of ether cleavage, metabolism of R-20458 by tissue homogenates illustrates the primary sites of metabolism which lead to the plethora of metabolites observed *in vivo*. The *in vitro* metabolism of R-20458 was approached by Gill *et al.* (1972, 1974) by basing identification of metabolites on cochromatography with a series of synthetic standards and on a series of reactions diagnostic for various functionalities (Hammock *et al.*, 1974b). The metabolism of the 2*Z* and the 2*E* isomers of R-20458 as well as further

metabolism of primary metabolites was investigated in addition to the olefinic precursor of R-20458 and juvenoids with *p*-nitrophenyl and methylenedioxyphenyl substituents in subcellular fractions of a variety of tissues from the rat, mouse, and rabbit with and without induction (Singh, 1973). The 6,7-epoxide, 2,3-olefin, and ethylphenoxy moieties were established as being labile to rapid hydrolytic and oxidative metabolism (Gill *et al.*, 1972, 1974). The 6,7-olefin of the diene (I) is much more rapidly epoxidized than the 2,3-olefin group. Epoxide hydration was observed in all hepatic subcellular fractions, but in contrast to investigations with other substrates (Jerina *et al.*, 1968; Oesch, 1973), much of the hydrolase activity was found in the 100,000 *g* soluble or cytosolic fraction (Gill *et al.*, 1972, 1974). Hammock *et al.* (1976) compared the metabolism of R-20458, JH I, and Ro-8-4314 in the microsomal and cytosolic fractions of mouse liver and kidney and found that the cytosolic fraction made a significant contribution to hydration and the relative rates of hydration were R-20458 > JH I > Ro-8-4314 (Figures 1.1 and 1.2). Mumby and Hammock (1979a, b, c) synthesized and monitored the relative initial rates of hydration of a number of juvenoids by the cytosolic fraction. Gill and Hammock (1979, 1980) further investigated the properties of the enzyme in several tissues of four mammals using R-20458 and *cis*- and *trans*-methylepoxystearates where substantial activity was additionally found in the mitochondrial fraction. Subsequently, the effect of this enzyme system has been studied with a variety of substrates (Hammock *et al.*, 1980a, b; Ota and Hammock, 1980). With the related methoxy and ethoxy juvenoids, *O*-dealkylation replaces epoxide hydration as a major route of metabolism in mouse liver microsomes (Figure 1.6) (Hammock *et al.*, 1975a).

Using isolated rat hepatocytes and two forms of non-induced rat hepatic cytochrome P450 (P451) in a reconstituted system, Agosin *et al.* (1979) and Morello and Agosin (1979) studied the metabolism of R-20458, methoprene, hydroprene, and JH I. They found rapid conversion of R-20458 to the α-hydroxyethylphenoxy derivative by both forms of P450. These workers also demonstrate reasonable proof for glutathione-*S*-epoxide transferase activity in rat hepatocytes using JH. Although one would expect such a reaction to occur, Gill *et al.* (1974) did not find extensive evidence for glutathione conjugation using R-20458 as could be predicted for trisubstituted epoxides (Chasseaud, 1979). However, the conjugated 2,3-olefin groups of JH, methoprene, and hydroprene will chemically react with glutathione (Sparks and Hammock, 1980a) as expected since an analogous reaction with thiophenol is used in the synthesis of methoprene and hydroprene (Siddall, 1976).

Toxicology

Juvenile hormone (Siddall and Slade, 1971) and two early epoxy amide and dichloro amide juvenoids (Cruickshank, 1971) demonstrated very low toxicity to rodents. Furthermore, it appears that recent toxicological studies with

the terpenoid phenoxy ether juvenoids have been generally favourable. Pallos *et al.* (1971) report negligible acute toxicity for R-20458. A series of studies was carried out on the toxicology of R-20458 to cattle, sheep, and swine following dermal spray or oral administration; blood chemistry and numerous other parameters were monitored in the test animals. Even at very high doses no effects of R-20458 were noted in swine or cattle, but leucopenia in sheep and testicular atrophy in rams were noted. A more highly purified technical sample failed to demonstrate the above symptoms when retested. No teratogenicity or toxicity was observed following intraperitoneal injection of R-20458 to hamsters on day 8 of gestation (Smalley *et al.*, 1974; Wright, 1976; Wright and Smalley, 1977). From a biochemical perspective, it was found that R-20458, the corresponding diene (I) and diol (K) at 0.1–2 mM, exhibit a concentration dependent inhibition of O_2 uptake by rat liver mitochondria (Hammock, 1973), and a similar observation was made with JH using mitochondria from the Indian meal moth, *Plodia interpunctella* (Firstenberg and Silhacek, 1973). It is unlikely that effects at such high concentrations represent a toxicological risk to man or a JH mode of action in insects, and the effects are probably related to the hydrophobicity of the compounds involved.

A number of juvenoids and, of course natural JH, contain the epoxide functionality. As highly strained ethers, epoxides are electrophilically reactive and some epoxides may alkylate biological materials including proteins and nucleic acids (Miller and Miller, 1977). In order to be a potent mutagen, such a compound must (1) readily alkylate biological material; (2) have an affinity for nucleic acids; and (3) either be stable enough to reach a critical site or be formed at the site. The extraordinary stability of the trisubstituted alkyl epoxides present in JH and juvenoids to acid hydration was first demonstrated by the rigorous conditions necessary to form the corresponding diols (Gill *et al.*, 1972; Slade and Zibitt, 1972). Although such epoxides can be detected in solution or on paper or thin layer chromatograms with 4-(*p*-nitrobenzyl) pyridine (selective for alkylating agents) (Hammock *et al.*, 1974c), juvenoids are much less reactive with this reagent than epoxides known to mutagenic (Hammock, unpublished). In general, monosubstituted alkyl epoxides are much less mutagenic than the corresponding disubstituted epoxides (El-Tantawy and Hammock, 1980, and included references), and Ivie *et al.* (1980b) demonstrated that trisubstituted psoralen glycidyl ethers were, at best, very weak mutagens, while the corresponding monosubstituted compound demonstrated the highest mutagenicity reported for any alkyl epoxide in the Ames' *Salmonella* system (Ames *et al.*, 1975). The same paper again illustrated the importance in mutagenicity of a group with high affinity for DNA or perhaps a group that can intercalate with it. In a screen of JH, geranyl phenoxy ether juvenoids, and a series of metabolites including the diepoxide (F), the 2,3-epoxide, and the diol-epoxide (G), no mutagenicity was detected in the *Salmonella* system (Hammock, unpublished). One possible risk from such juvenoids is that they appear to inhibit the hydration of some epoxides

by the mammalian cytosolic hydrolase (Hasegawa and Hammock, unpublished), and another possibility is that esters of the α-hydroxyethylphenoxy derivatives (Figure 1.5) might be alkylating agents (Miller and Miller, 1977), but there is no evidence for a problem with these metabolites. However, even the most stable juvenoids are so biodegradable that such inhibition is probably a laboratory curiosity. Although no compound can be proven safe, the available data indicate that mutagenic risk from those juvenoids tested is minimal.

R-20458 and, to a lesser extent, other phenyl ethers are toxic to algae and retard algal growth. However, these effects are seen at such high concentrations that it is unlikely that the use of these compounds in reasonable insect-control programmes would present a significant risk (Gill *et al.*, 1972, 1974; Hammock, 1973). The juvenoids so far examined seem to present minimal acute risk to aquatic invertebrates. For instance, R-20458, to the limit of its solubility, caused no 48 h toxicity to the marine isopod *Sphaeroma quoyanum* or its commensal *Iais californica*, but it has profound effects on sexual dimorphism in the terrestrial isopod, *Porcellio scaber* (Rotramel and Hammock, unpublished). Since juvenoids may disrupt the development of arthropods, careful studies are needed, but there is no indication, to date, that juvenoids present a substantially greater risk to most non-target arthropods than do classical pesticides (Breaud *et al.*, 1977; Costlow, 1977).

Norris *et al.* (1974) report on some rodent studies with 4-(4,8-dimethyldecyloxy)-1,2-(methylenedioxy)benzene. The acute oral LD_{50} was $>4\,g\,kg^{-1}$ and long term feeding studies were carried out at $1–100\,mg\,kg^{-1}\,day^{-1}$ while monitoring numerous parameters. Toxicological problems appeared minimal with neonate survival and growth, and litter size decreased at $100\,mg\,kg^{-1}\,day^{-1}$ but not at lower levels. Unsworth *et al.* (1974) reported that a methylenedioxyphenoxy juvenoid was teratogenic to mice at $1\,mg\,kg^{-1}$ while several very closely related compounds (Figure 1.2) showed no effects. The juvenoid Ro-10-3108 and its major metabolites show little if any acute toxicity to mice (LD_{50} $>5\,g\,kg^{-1}$), and following a 96 h exposure Ro-10-3108 demonstrated LC_{50}s of >5000 p.p.m. to three fish species (Dorn *et al.*, 1976). A tabular summary of the toxicology of Ro-10-3108 has been given by Zurflüh (1976).

Environmental Fate

The first reports on the environmental degradation of juvenoids were those of Gill *et al.* (1972) on R-20458 (Figure 1.2) and Pawson *et al.* (1972) on JH I and a methylenedioxyphenoxy juvenoid. Gill *et al.* (1972) reported slow decomposition on silica gel plates exposed to sunlight unless photosensitizers were present. Rapid decomposition then occurred especially at the benzylic position yielding α-hydroxyethylphenoxy and acetophenoxy derivatives (Figure 1.5). Pawson *et al.*

(1972) report rapid degradation of both compounds (and loss of biological activity) when exposed to ultraviolet light as thin films on glass. Numerous photodegradation products were formed including the corresponding diepoxides (F).

Much of the subsequent work on environmental degradation of such compounds has been reviewed by Schooley and Quistad (1979). Gill *et al.* extended their preliminary study on R-20458 in a more comprehensive 1974 report. Photodecomposition of the epoxide R-20458 (J), diene (I), and diol (K) were compared on silica gel chromatoplates exposed to natural sunlight, a sunlamp, or u.v. lamp. R-20458 is very stable on silica gel in the dark, it decomposes at relatively low identical rates which are similar with sunlight or sunlamp exposure, but at a high rate with u.v. exposure. The rate curves are definitely phasic with all three light sources. U.v. irradiation resulted in highly polar products which failed to move from the origin of a t.l.c. plate in several systems, while sunlight led to products of intermediate polarity. Twenty-two candidate triplet sensitizers were screened, most of which enhanced the photochemical degradation of R-20458. No correlation could be drawn between the triplet energy (39–74 kcal mol^{-1}) and the rate of R-20458 degradation. The most active sensitizer was anthracene which, on exposure to sunlight, converted R-20458 to very polar products reminiscent of u.v. exposure. Dyes generally failed to sensitize decomposition, and rotenone, which is a very potent sensitizer for dieldrin (Ivie and Casida, 1971) failed to sensitize R-20458 degradation (Singh, 1973).

A comparison of the stabilities of R-20458 and the corresponding methoxide and ethoxide (Figure 1.6) when exposed to sunlight on silica gel demonstrated that the epoxide was only slightly less stable than the alkoxides. Since epoxide hydration and rearrangement is much less important than other photodecomposition pathways, such a result could be anticipated (Hammock *et al.*, 1975a).

On silica gel, benzylic oxidation of R-20458 to α-hydroxyphenoxy and acetophenoxy derivatives was a major route of degradation. Rates of epoxide hydration to the diol (K, Figure 1.4) were somewhat variable and, as expected, they increased under moist conditions. Epoxidation at the 2,3-olefin yields the diepoxide (F) and a variety of cyclic (A–D) and bicyclic ethers (not shown). The diol was subsequently oxidized to the hydroxyketone (P), and either the epoxide, diene, or diol, depending on conditions, may yield the allylic alcohol (O). The diene (I) was converted to the epoxide (J) on silica gel in the dark and at a much higher rate upon exposure to light, while the diepoxide (F) yielded largely rearrangement products (A–D) discussed earlier. In all compounds, ether cleavage was evidenced by the presence of free phenols and, in all cases, several unknown photoproducts were detected. As discussed earlier, photodegradation products had greatly reduced biological activity (Singh, 1973; Gill *et al.*, 1974; Hammock *et al.*, 1974a).

Photoalteration of the diene (I), R-20458 (J), and diol (K) at 0.5 p.p.m. in distilled water was also studied following exposure to sunlight or a sunlamp. As on silica gel, the diene was much less stable than the epoxide or diol, giving a variety of photoproducts including the allylic alcohol (O). Peroxides may be involved in this pathway (Hammock, 1973). Benzylic oxidation, 2,3-epoxidation and cyclization, epoxide hydration, and ether cleavage again occurred (Singh, 1973; Gill *et al.*, 1974).

The photodecomposition of the diene (I), R-20458, and diol (K) was also studied in monoaxenic cultures of living or dead *Chlorella* sp. and *Chlamydomonas teinhardii* and compared with decomposition in chlorophyll solutions. Epoxide hydration was a major route of metabolism in *C. teinhardii* while *Chlorella* sp. demonstrated a high rate of benzylic oxidation. With culture medium, chlorophyll solutions, or dead algae, the diepoxide (F) was a predominate metabolite. With the algae, especially *Chlorella,* surprisingly large amounts of phenolic and acidic metabolites formed (Hammock, 1973; Gill *et al.*, 1974).

Hangartner *et al.* (1976) reported a comparison of the stability of several juvenoids on silica gel irradiated with a high intensity sunlamp, in pukka water under field conditions, in water at pH 4, and on bean leaves. Ro-10-3108 (Figure 1.2) was significantly more stable than a variety of other juvenoids. The methylenedioxyphenyl group of a related juvenoid was very unstable photochemically and hydrolytically. Elimination of the 2,3-olefin of R-20458 definitely increased the photochemical stability of the resulting compound. Surprisingly, methylethyl rather than dimethyl substituents on the epoxide also increased the stability. This observation was further investigated by Mumby and Hammock (1979b) by comparing the stability of 17 juvenoids to aqueous acid. The influence of electronic substituent effects could be predicted from the classic studies of Pritchard and Long (1956; Long and Pritchard, 1956), but an increase in only the hydrophobicity of the epoxide substituents (dimethyl to methylpropyl) could increase the stability *c.* 9 times and the biological activity on *T. molitor c.* 50 times. The enhanced stability of Ro-10-3108 over R-20458 and methoprene could clearly be advantageous for some applications (Hargartner *et al.*, 1976) and this approach could possibly still be taken further with compounds such as those described by Schwarz *et al.* (1974) (Figure 1.2). The stability predicted in the laboratory for Ro-10-3108 was demonstrated under field conditions on the summer fruit tortrix moth (*Adoxophyes orana*), the San José scale (*Quadraspidiotus perniciosus*), and the citrus snow scale (*Unaspis citri*).

Dorn *et al.* (1976) investigated the degradation of Ro-10-3108 in polluted water held under field conditions. The major routes of degradation included benzylic oxidation (Figure 1.5), ether cleavage, epoxide hydration (K) (Figure 1.4), and conversion of the resulting diol to two isomeric allylic alcohols analogous to product O (Figure 1.4). The metabolites all had reduced biological activity, although the α-hydroxyphenoxy and acetoxyphenoxy analogues of

Ro-10-3108 are still active juvenoids. Dorn *et al.* (1976) attribute the limited number of degradation products in Ro-10-3108 relative to R-20458 to the absence of the biodegradable 2,3-olefin.

ANTI-JUVENILE HORMONES

The theoretical attraction of anti-juvenile hormones (anti-JHs) as insect control agents has stimulated work in numerous laboratories for several years. It was Bowers (1976) who transformed speculation into reality with the announcement of the structures of the precocenes. One could obtain anti-JH effects by inhibiting JH production or release, disrupting JH transport, stimulating JH degradation, blocking JH action at a target site, or subtly disrupting insect regulation. The search for such compounds has involved screening of natural and man-made products, investigating insect–plant or insect–insect interaction, or attempting to disrupt known biosynthetic pathways. To date only two series of compounds have been described in the literature which demonstrate clear anti-JH activity (i.e. precocenes and ETB).

Ethyl 4-[2-(*tert*-butylcarbonyloxy)]butoxybenzoate (ETB) causes black pigmentation in *Manduca sexta* larvae, an effect which is alleged to indicate JH deficiency at a level not severe enough to produce premature metamorphosis (Staal, 1977). The mode of action of ETB is largely unknown, but it is a weak JH esterase inducer in *T. ni* and it appears to act as a JH agonist/antagonist (Sparks *et al.*, 1979b).

Precocene—Mode of Action

Much of the information concerning the mode of action of precocene II comes from work with *Oncopeltus fasciatus*. Precocene II inactivates the corpora allata (Masner *et al.*, 1979) which lose the ability to secrete JH (Müller *et al.*, 1979). The effectiveness of precocene II is dependent on the timing of application and maximal reponses are obtained if treatment occurs when the corpora allata are active (Masner *et al.*, 1979; Unnithan and Nair, 1979). When precocene II is applied to certain aged larvae or 1–7 day old adults, the insects are rendered sterile (Müller *et al.*, 1979). Ultrastructural examination of the corpora allata of *O. fasciatus* treated with precocene II as young adults revealed that the glands consisted of immature cells (Liechty and Sedlak, 1978) which implied lack of differentiation of tissue.

Schooneveld (1979a, b) has shown that precocene II induces collapse of the corpora allata of nymphal *Locusta migratoria*. Necrosis of cells is followed by phagocytosis of cell fragments by haemocytes. Hence, *precocene II is cytotoxic*. Brooks *et al.* (1979a, b) have postulated that the cytotoxicity of precocene II in *L. migratoria* results from an activated alkylating agent such as the 3,4-epoxide of precocene II. Support for this hypothesis is based partly on the known high

chemical reactivity of this 3,4-epoxide (Jennings and Ottridge, 1979) and also on ıe results of Brooks *et al.* (1979a, b) which demonstrate that the corpora allata metabolize precocene II cleanly to the 3,4-dihydrodiol (presumably via the 3,4-epoxide).

Precocene II is also known to inhibit vitellogenesis in *O. fasciatus* (Masner *et al.*, 1979) and *D. melanogaster* (Landers and Happ, 1979), but it is not antigonadotropic in the adult female *A. aegypti*, a morphogenetically insensitive insect (Kelly and Fuchs, 1978). Precocene II also interferes with sex attractant production in the brown cockroach (Burt *et al.*, 1979).

Insect Metabolism of Precocene II

Nine insect species showed a 37-fold variation in the rate of precocene II metabolism *in vivo* (Ohta *et al.*, 1977). The principal metabolite in each insect was a 3,4-diol (Figure 1.7) which allegedly arose from hydration of a 3,4-epoxide. This 3,4-diol is also the major aglycone from metabolism of precocene II by the brown cockroach (*Periplaneta brunnea*) (Burt *et al.*, 1979). Although Ohta *et al.* (1977) claimed to have synthesized the 3,4-epoxide as an authentic standard, the structure of their product was subsequently disputed (Bergot *et al.*, 1980; Soderlund *et al.*, 1980). More recently using carefully controlled conditions the preparation of authentic 3,4-epoxide has been reported, but it is readily evident that this epoxide is chemically quite reactive and unstable (Jennings and Ottridge, 1979; Soderlund *et al.*, 1980). Most workers agree that the 3,4-epoxide metabolite probably plays a key role in solving questions about the precocene mode of action, but thus far the 3,4-epoxide metabolite itself has not been onclusively identified.

A comparison of different tissues showed that the fat bodies of both *Trichoplusia ni* and *Ostrinia nubilalis* fifth instar larvae possessed high *in vitro* metabolic activity toward precocene II (Burt *et al.*, 1978). Although these lepidopterans are insensitive to the morphogenetic effects of precocene, Burt *et al.* (1978) studied the effects of suspected epoxide hydrolase inhibitors on precocene metabolism in fat body homogenates from these insects. Inhibition of precocene metabolism could be shown with all five inhibitors used, but accumulation of intermediate metabolites (e.g. 3,4-epoxide) could not be shown. A 3-hydroxy hydration metabolite was shown as a major product in *T. ni* homogenates (Ohta *et al.*, 1977).

Bergot *et al.* (1980) compared the metabolism of precocene II in several insect species which showed varying response to its morphogenetic effects. By using a treatment method (topical) and dose rate appropriate for inducing the desired morphological reponse in sensitive insects, Bergot *et al.* (1980) hoped to detect an activated metabolite, if such a compound existed. The metabolites in insect haemolymph were strikingly different from previous results reported for fat body

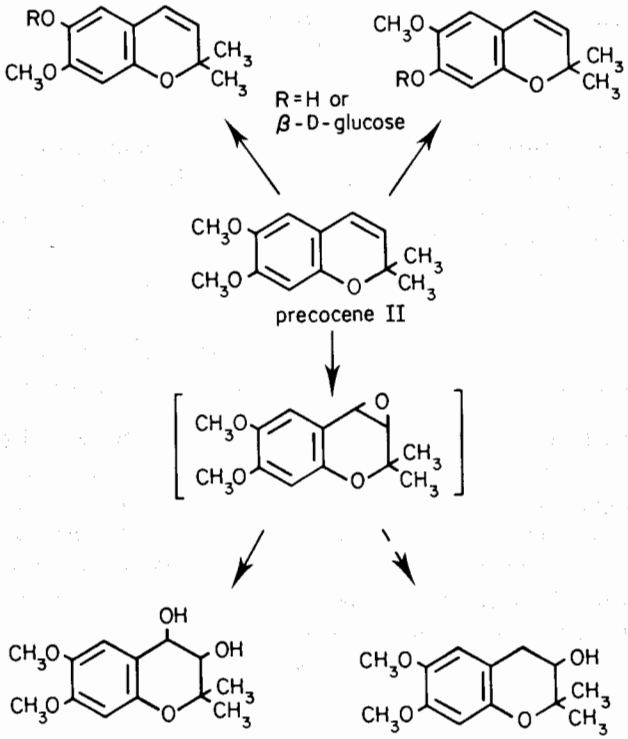


Figure 1.7 Insect metabolism of precocene II

homogenates. The primary metabolites in haemolymph were glucosides of *O*-demethylated precocene (Figure 1.7). Since these *O*-β-glucosides of 6- and 7 monomethylated precocene II were demonstrated in both sensitive and insensitive species, no evidence was found for a haemolymph-borne, biologically effective 'activated metabolite'. If such a biologically active product exists, it is likely produced *in situ* at the target tissue (Bergot *et al.*, 1980). Although Bergot *et al.* (1980) were unable to detect free phenolic metabolites in haemolymph, Soderlund *et al.* (1980) using *T. ni* fat body homogenates were able to detect the corresponding 6- and 7-desmethylated precocenes as free metabolites. They also recovered the 3,4-diol metabolites as a mixture of *cis* and *trans* isomers. A summary of the insect metabolites of precocene is given in Figure 1.7.

BENZOYLPHENYL UREAS—MODE OF ACTION

History

The mode of action of benzoylphenyl ureas has been intensely investigated over the last decade (for reviews see Post and Mulder, 1974; Marx, 1977; Verloop

and Ferrell, 1977; Ker, 1978). The insecticidal activity of benzoylphenyl ureas was discovered at Philips-Duphar (The Netherlands) originally with DU-19111 (Figure 1.8) which was synthesized as an analogue of dichlobenil, a pre-emergence herbicide. It became visibly evident that insect larval death was invariably connected with the moulting process (Post and Mulder, 1974).

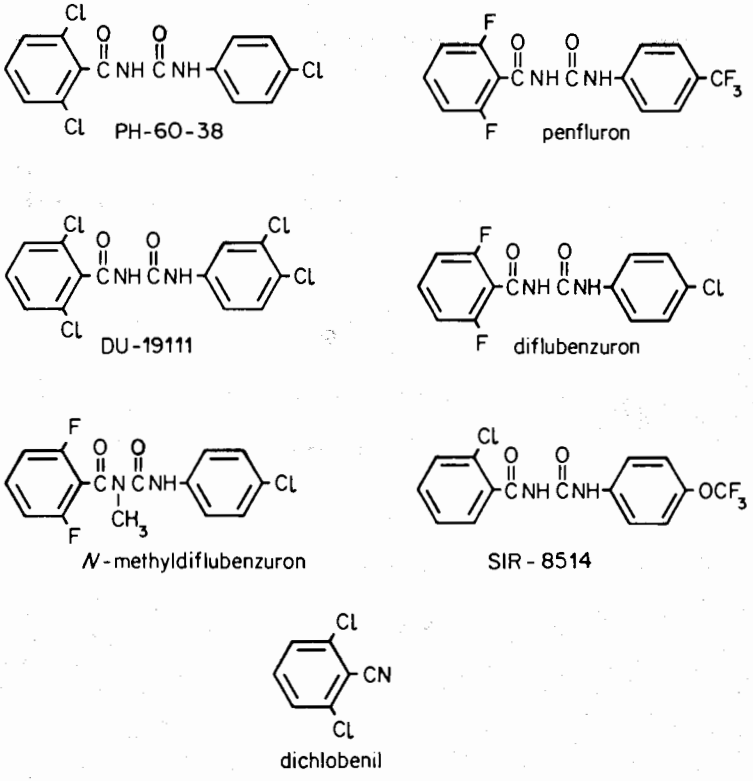


Figure 1.8 Benzoylphenyl ureas and related compounds

Although several alternative theories of the mode of action for this new class of compounds arose subsequently, the initial report implicating chitin inhibition as a prelude to insect mortality also came from Philips-Duphar (Post and Vincent, 1973). Using DU-19111, Post and Vincent (1973) showed the benzoylphenyl urea prevented incorporation of [^{14}C]glucose into the cuticular chitin of *Pieris* larvae. In the process of optimizing the insecticidal activity of DU-19111 by analogue synthesis, diflubenzuron arose as the leading candidate for commercialization. Diflubenzuron has been shown to inhibit chitin biosynthesis in a number of insects: *Lymantria dispar* (Salama *et al.*, 1976), *Leptinotarsa* (Post and Vincent, 1973; Grosscurt, 1977, 1978), *Oncopeltus fasciatus* (Hajjar and Casida, 1978, 1979), *Pieris brassicae* (Mulder and Gijswijt, 1973; Post *et al.*, 1974; Deul *et al.*,

1978). *Culex pipiens* (Hajjar, 1979), locusts (Hunter and Vincent, 1974), *Locusta migratoria* (Clarke *et al.*, 1977), *Schistocerca gregaria* (Ker, 1977), *Chil suppressalis* (Nishioka *et al.*, 1979), and *Musca domestica* (Ishaaya and Casida, 1974).

Inhibition of Chitin Biosynthesis

Many workers have investigated the effects of diflubenzuron on the biosynthetic pathway leading to chitin in order to ascertain exactly which step is inhibited (Figure 1.9). Most evidence concerning inhibition of chitin biosynthesis suggests that chitin synthetase is the key enzyme. Early work with larval *P. brassicae in vitro* showed that DU-19111 prevented conversion of [^{14}C]glucose to [^{14}C]chitin with an attendant accumulation of *N*-acetylglucosamine (Post *et al.*, 1974). Although *N*-acetylglucosamine is not an intermediate in chitin biosynthesis, these authors proposed that DU-19111 partially blocked the process

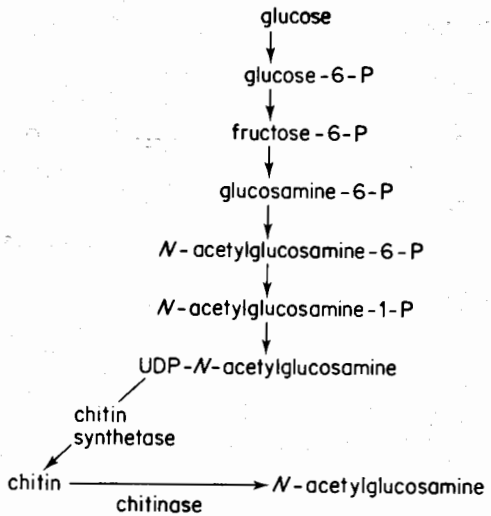


Figure 1.9 Biosynthetic production of chitin

by which *N*-acetylglucosamine units added to the growing chitin polymer. The same group (Post *et al.*, 1974) found that diflubenzuron completely blocked chitin formation and uridine 5′-diphospho-*N*-acetylglucsoamine (UDPAG) accumulated, suggesting inhibition of chitin synthetase. UDPAG also accumulates upon diflubenzuron inhibition of chitin biosynthesis in *O. fasciatus* (Hajjar and Casida, 1978, 1979) and *M. domestica* (van Eck, 1979). Hence, it appears that the most experimentally supported explanation is that the *primary mode of action of diflubenzuron in insects is prevention of chitin biosynthesis via chitin synthetase inhibition,* although some workers recommend caution in

accepting this explanation (Mayer *et al.*, 1980). Proof that chitin synthetase is the target for benzoylphenyl ureas awaits isolation of the enzyme from insect tissues for *in vitro* analysis, but enzyme purification heretofore has been elusive (van Eck, 1979; Hajjar and Casida, 1979).

The morphological manifestation of diflubenzuron treatment of insects is an imperfect cuticle which is particularly noticeable at the time of ecdysis. The endocuticle contains reduced levels of chitin while epicuticular and exocuticular tissues (relatively chitin-free) are unaffected (Mulder and Gijswijt, 1973). The impaired attachment of endocuticle to the epidermis (Mulder and Gijswijt, 1973; Salama *et al.*, 1976) and lower chitin content confer reduced cuticular rigidity (Hunter and Vincent, 1974). Hence, insect mortality results from cuticular malformation.

A useful byproduct of investigations of the mode of action of benzoylphenyl ureas has been the development of a number of systems for the bioassay of potential chitin synthesis inhibitors (Table 1.5). A very sensitive *in vitro* system was developed using cockroach leg regenerates (Marks and Sowa, 1974; Sowa

Table 1.5 Assay of chitin biosynthesis

Precursor	Insect	Type	Reference
D-[6-^{14}C]glucose	*Pieris*, larvae	*in vivo*	Deul *et al.*, 1978
[6-^{3}H]glucosamine	*Musca*, larval body walls	*in vitro*	van Eck, 1979
[acetyl-^{14}C]*N*-acetylglucosamine [*U*-^{14}C]glucose [*U*-^{14}C]glucosamine	*Oncopeltus*, adult abdomens	*in vitro*	Hajjar and Casida, 1979
[*U*-^{14}C]glucose	*Oncopeltus*, larvae	*in vivo*	Hajjar and Casida, 1979
[^{14}C]*N*-acetyl glucosamine [^{14}C]glucosamine	*Leucophaea*, nymphal leg regeneration	*in vitro*	Marks and Sowa, 1974 Sowa and Marks, 1975 Cohen and Marks, 1979
D-[6-^{3}H]glucose	*Plodia*, larval wing discs	*in vitro*	Oberlander and Leach, 1974
None, cuticle thickness measured	*Chilo*, diapausing larvae	*in vitro*	Nishioka *et al.*, 1979
[^{14}C]acetylglucosamine D-[^{14}C]glucosamine D-[^{14}]glucose D-[^{14}C]fructose	*Stomoxys*, imaginal pupal tissue	*in vitro*	Mayer *et al.*, 1980

and Marks, 1975). In this system diflubenzuron inhibited chitin synthesis with an I_{50} of 6×10^{-10} M, but unfortunately the assay takes 2 weeks and requires β ecdysone activation. By tissue culturing of integumentary cuticle of the rice stem borer (*Chilo suppressalis*) and then measuring cuticle thickness after exposure to potential inhibitors Nishioka *et al.* (1979) showed a respectable 6×10^{-7} M I_{50} for PH 60–38 (the dichlorobenzamide analogue of diflubenzuron, Figure 1.8). In the *in vivo* assay of Deul *et al.* (1978) virtually complete inhibition of chitin biosynthesis was demonstrated 15 min after application of diflubenzuron (1 μg/*Pieris* larva). One of the better *in vitro* bioassays was developed by Hajjar and Casida (1978, 1979) using the isolated abdomens of readily available *O. fasciatus*. This convenient chitin-synthetisizing system showed good structure activity correlations for 24 diflubenzuron analogues in comparing toxicity with chitin inhibitory activity. Diflubenzuron gave an I_{50} of 6×10^{-7} M (0.25 μg g^{-1} of abdomen) when [^{14}C]glucose was used as substrate. Using *M. domestica* larval body walls van Eck (1979) reported a 10-fold increase in sensitivity over the Hajjar–Casida system ($I_{50} = 0.03$ μg g^{-1} tissue). Hence, the target for diflubenzuron in house flies is apparently more sensitive than that of milkweed bugs.

Secondary Effects

An alternative proposal for the mode of action of diflubenzuron is that it impedes metabolism of ecdysone (Yu and Terriere, 1975b, 1977c). The observed morphological deformities after treatment with diflubenzuron could then result from increased levels of chitinase and phenol oxidase which has indeed been shown in *M. domestica* (Ishaaya and Casida, 1974). However, subsequent data refute this hypothesis as the mode of action for diflubenzuron. Deul *et al.* (1978) found that neither DU-19111 nor diflubenzuron has any effect on chitinase activity in *P. brassicae* either *in vitro* or *in vivo*. Hajjar and Casida (1979) provided the most conclusive evidence that diflubenzuron does not alter *in vivo* metabolism of either α- or β-ecdysone by fifth instar milkweed bug nymphs nor does it alter the endogenous titre of β-ecdysone in pharate *Stomoxys calcitrans* pupae (O'Neill *et al.*, 1977). The juvenoid R-20458 does not synergize diflubenzuron activity nor does β-ecdysone affect diflubenzuron activity in mosquitoes and milkweed bugs (Hajjar, 1978).

In general, diflubenzuron does not affect protein synthesis in insect cuticle. Protein synthesis is unaffected in the cuticle of *Leptinotarsa* (Post and Vincent, 1973), *P. brassicae* (Hunter and Vincent, 1974; Post *et al.*, 1974), *Anthonomus grandis* (Mitlin *et al.*, 1977), or *Leucophaea maderae* leg regenerates (Marks and Sowa, 1974). Ishaaya and Casida (1974) reported a dose dependent increase in the protein : chitin ratio when larval house fly cuticle was treated with diflubenzuron, an alteration which they suggest affects the elasticity and firmness of endocuticle. Contrary to this finding, Clarke *et al.* (1977) reported that

diflubenzuron treatment of the peritrophic membrane of *Locusta* resulted in a constant ratio of protein : chitin, both of which were individually reduced. The constancy of the protein : chitin ratio may be limited to cuticles which are not covalently cross-linked since deposition of protein in sclerotized areas is largely unaffected (Clarke *et al.*, 1977). Although the protein : chitin ratio in the peritrophic membrane of adult *Calliphora* was not determined, diflubenzuron treatment decreased chitin levels as well as decreasing the mass and length of peritrophic membrane (Becker, 1978). Grosscurt (1978) showed that diflubenzuron modified the mechanical penetrability of adult *L. decemlineata* elytra, an effect which paralleled the kinetics of inhibition of chitin formation. Grosscurt concluded that effects on penetrability were due to interference of diflubenzuron with chitin–protein bonding in the elytra.

Several miscellaneous aspects of the mode of action of diflubenzuron have been explored. In locust species diflubenzuron has no effect on cuticular tanning (Hunter and Vincent, 1974; Ker, 1977). It also has no effect on transport of [^{14}C]glucose and its metabolites into the integument of *C. pipiens* (Hajjar, 1978; Hajjar and Casida, 1979). Although Salama *et al.* (1976) reported that diflubenzuron did not affect internal tissue or spermatogenesis in lepidopterous insects, Mitlin *et al.* (1977) found effects on lipoprotein synthesis and inhibition of testicular growth in male *A. grandis*. The diminishment of sexual function was attributed in part to inhibition of DNA synthesis by diflubenzuron. Interestingly, although diflubenzuron was originally the result of a probe for herbicidal activity, it has little effect on plants. Diflubenzuron has no effect on photosynthesis or leaf ultrastructure in soy beans and respiration is only stimulated in a transitory manner at high rates (Hatzios and Penner, 1978). Unlike Polyoxin D which inhibits chitin biosynthesis in both fungi and insects, benzoylphenyl ureas block this synthesis only in insects (van Eck, 1979). However, Gijswijt *et al.* (1979) have shown that for *P. brassicae* the inhibition of cuticle deposition and of chitin synthesis appears to be the same for both Polyoxin D and diflubenzuron.

METABOLISM OF DIFLUBENZURON AND RELATED COMPOUNDS

Insect Metabolism

In general, diflubenzuron is refractory to degradation by most insects studied to date. Minimal metabolism of diflubenzuron is reported for *Estigmene acrea* (salt marsh caterpillar; Metcalf *et al.*, 1975; Verloop and Ferrell, 1977), *Culex pipiens* (mosquito; Metcalf *et al.*, 1975), and *Pieris brassicae* (cabbage butterfly; Verloop and Ferrell, 1977). *Musca domestica* (house fly) degrades diflubenzuron more effectively than *Stomoxys calcitrans* (stable fly), but even *M. domestica* only metabolized about 10% of the applied dose after topical application (Ivie and Wright, 1978). Reduced penetration coupled with more efficient metabolism and rapid excretion are important factors in explaining resistance to diflubenzuron in

M. domestica (Pimprikar and Georghiou, 1979). An unnatural method of application (i.e. injection) resulted in only 21% degradation of diflubenzuron by *M. domestica* after 3 days (Chang, 1978).

The metabolism of diflubenzuron has been most extensively studied in the boll weevil (*Anthonomus grandis*). Although initial reports indicated essentially no degradation by this insect (Still and Leopold, 1975, 1978; Verloop and Ferrell, 1977), more recent data (Chang and Stokes, 1979) demonstrated up to 23% degradation after 4 days. Much interest has focused on the transfer of diflubenzuron from treated to untreated weevils (Moore *et al.*, 1978) and the secretion of unmetabolized compound into eggs which is responsible for inhibition of hatching (Bull and Ivie, 1980). The metabolites of diflubenzuron reported from insects are given in Table 1.6, and the metabolic fate of diflubenzuron has also been discussed by Sparks and Hammock (1980a).

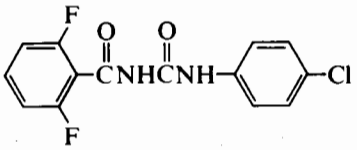
Mammalian Metabolism and Toxicology

Hydrolysis and aromatic hydroxylation are the two primary metabolic pathways in rats (Verloop and Ferrell, 1977). About 20% of the diflubenzuron was hydrolysed by rats to 2,6-difluorobenzoic acid and 4-chlorophenylurea. The isolation of considerably less than stoichiometric amounts of 4-chlorophenylurea suggested that it is further degraded. Aromatic hydroxylation of diflubenzuron at both phenyl rings contributed 80% of the metabolic degradation. These hydroxylated metabolites represented almost all of the biliary ^{14}C products and about half of those in urine. Hydroxylated diflubenzurons are devoid of insecticidal activity on *A. Grandis* (Bull and Ivie, 1980) and are readily excreted by rats if given orally (Ivie, 1978).

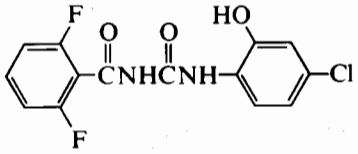
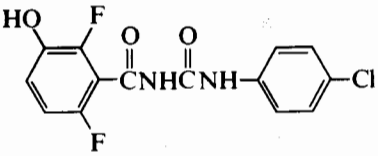
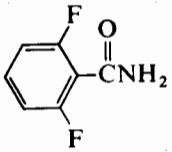
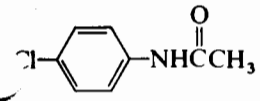
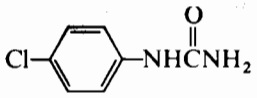
The fate of diflubenzuron has been studied comprehensively in sheep and cattle (Ivie, 1977, 1978). Although sheep and cattle metabolized diflubenzuron in a qualitatively similar manner (Table 1.7), the major sheep metabolites arose from cleavage of the amide bond by hydrolysis whereas, in the lactating cow, metabolic transformation resulted primarily from hydroxylation at the 3-position of the 2,6-difluorobenzoyl moiety. The fate of the 4-chlorophenyl ring was largely undetermined since 4-chlorophenylurea was the single such metabolite identified and it was recovered in low yield. In both castrate male sheep and a lactating cow dosed orally, diflubenzuron was extensively metabolized and almost totally excreted. Cannulation of the bile duct in sheep demonstrated the importance of biliary excretion while minor levels of radiolabelled metabolites were secreted into milk.

Diflubenzuron has low acute mammalian toxicity (Ferrell and Verloop, 1975) with an acute LD_{50} in rats of 4600 mg kg^{-1} (Lewis and Tatken, 1979). Holstein bull calves can consume up to 1 mg kg^{-1} per day of diflubenzuron without affecting growth or organ histopathology (Miller *et al.*, 1979). *In vitro* studies with rat C6 glial cells demonstrated that diflubenzuron is neither cytotoxic nor

Table 1.6 Insect metabolites of diflubenzuron



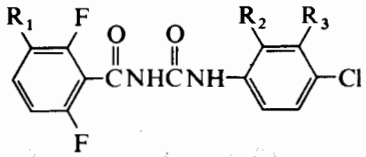
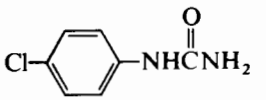
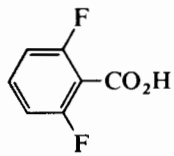
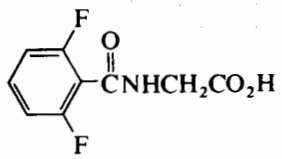
diflubenzuron

Metabolite	Insect (% applied dose)	Reference
	Boll weevil[a] House fly (12)	Chang and Stokes, 1979 Chang, 1978
	Boll weevil[a]	Chang and Stokes, 1979
	Boll weevil (2) Stable fly (0.8) House fly (0.3)	Chang and Stokes, 1979; Bull and Ivie, 1980 Ivie and Wright, 1978 Ivie and Wright, 1978 Pimprikar and Georghiou, 1979
	Stable fly (<0.2)	Ivie and Wright, 1978
	Stable fly (0.5) House fly (0.3)	Ivie and Wright, 1978 Ivie and Wright, 1978

[a] Hydroxylated diflubenzurons were detected only as conjugates, collectively representing up to 19% of the applied dose.

does it inhibit the synthesis of complex carbohydrates (glycosaminoglycans) in mammalian cells (Bishai and Stoolmiller, 1979). Using a *Salmonella* mutagenicity assay Seuferer *et al.* (1979) found that only 2,6-difluorobenzoic acid seemed mutagenic in bacteria (further testing showed this result to be a false positive) while diflubenzuron itself, 4-chlorophenylurea, 4-chlorophenol, and 4-chloroaniline were only borderline mutagens. Toxicology fears were somewhat ameliorated by recent data from the National Cancer Institute which partially absolve 4-chloroaniline from earlier allegations as a mammalian carcinogen

Table 1.8 Mammalian metabolites of diflubenzuron

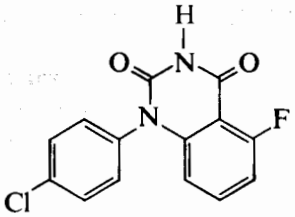
	Per cent total ^{14}C in excrement				
	Cow		Sheep		Rat
	urine	faeces	urine	faeces	
					
$R_1 = OH, R_2 = R_3 = H$	45	18	1	0.4	80
$R_1 = R_3 = H, R_2 = OH$	2	0.6	0.2	0.8	
$R_1 = R_2 = H, R_3 = OH$	4	0.7	0	0.4	
	0.6	—	0	—	2
	6	—	27	—	20
	7	—	22	—	—

(National Cancer Institute, 1979). Diflubenzuron is essentially non-toxic to fish (McKague and Pridmore, 1978) although 4-chloroaniline is relatively more toxic to bluegill (Julin and Sanders, 1978).

Environmental Fate

Schaefer and Dupras (1976, 1977) found that the environmental persistence of diflubenzuron in water appeared to be determined by the rate of hydrolysis and adsorption onto organic matter. High temperature and elevated pH enhanced instability. Schaefer and Dupras (1976) found minimal photodecomposition of diflubenzuron when a thin film on glass or a 0.1 p.p.m. aqueous solution was exposed to sunlight even though substantial photodegradation occurs when methanolic solutions are irradiated with artificial light sources (Ruzo *et al.*, 1974; Metcalf *et al.*, 1975). Ivie *et al.* (1979, 1980a) studied the fate of diflubenzuron in water and found a half-life of 56, 7 and < 3 days for pHs 4, 6, and 10, respectively.

The major degradation products in water were quantitated as a function of pH nd consisted of 4-chlorophenylurea, 2,6-difluorobenzoic acid, small amounts of 2,6-difluorobenzamide, and a unique quinazolinedione (4% yield after 56 days pH 10).



Quinazolinedione

The degradation of diflubenzuron by aquatic organisms has been reviewed by Schooley and Quistad (1979). Aquatic microbial metabolism has been reported by Metcalf *et al.* (1975), Schaefer and Dupras (1976, 1977), and Booth and Ferrell (1977). The degradation of diflubenzuron by fish, as well as the other components of an aquatic ecosystem, was detailed by Metcalf *et al.* (1975) and Booth and Ferrell (1977).

The degradation of benzoylphenyl ureas in soil was significant historically in the selection of diflubenzuron for commercial development (Verloop and Ferrell, 1977). Indeed an initial lead structure, the analogous 2,6-dichlorobenzamide (PH-60-38), was not pursued commercially because of its extreme persistence in agricultural soil (6–12 months). By replacing the two chlorines with fluorines an analogue (i.e. diflubenzuron) was found which was not only more susceptible to environmental degradation by hydrolysis, but also was unexpectedly more insecticidal. An important stability property of diflubenzuron was discovered from initial soil degradation studies, i.e. the rate of degradation was greatly dependent on particle size with smaller particles being degraded more rapidly. This axiom also extends to other degradation studies involving diflubenzuron including insect metabolism where topical application of organic solutions often results in deposits of large crystals which are more refractory to breakdown (Still and Leopold, 1978).

Diflubenzuron is still relatively persistent in soil. Metcalf *et al.* (1975) found virtually no degradation in soil after 4 weeks. When cotton plants previously sprayed with diflubenzuron were cultivated into soil, residues had not dissipated appreciably after 6 months (Bull and Ivie, 1978). Unmetabolized diflubenzuron represented 81% of the ^{14}C-residue with 2% as 4-chlorophenylurea the only identified metabolite. *The major metabolic pathway for diflubenzuron in soil involves hydrolysis to 2,6-difluorobenzoic acid and 4-chlorophenylurea* (Verloop and Ferrell, 1977; Mansager, *et al.*, 1979). Verloop and Ferrell (1977) found that up to 70% of the aniline-labelled diflubenzuron was recovered as 4-chlorophenyl urea which was relatively persistent whereas benzoyl-labelled

diflubenzuron gave only 20% as 2,6-difluorobenzoic acid, indicating greater environmental lability of this metabolite. By isolating 28% of the applie [^{14}C]diflubenzuron as $^{14}CO_2$ after 91 days Mansager *et al.* (1979) demonstrated that one or both of the aromatic rings can be exhaustively oxidized, albeit at a slow rate. These authors also found 4-chlorophenylurea as the major metabolite in soil (24% applied dose after 21 days).

Degradation of diflubenzuron in soil is primarily biological with little breakdown under sterile conditions (Verloop and Ferrell, 1977). Seuferer *et al.* (1979) have isolated four eucaryotic microorganisms from soil capable of degrading diflubenzuron. One species of *Fusarium* was capable of using diflubenzuron as a sole carbon source, producing 2,6-difluorobenzoic acid, 4-chlorophenylurea, 4-chloroaniline, 4-chloroacetanilide, acetanilide, and 4-chlorophenol as metabolites. Seuferer *et al.* (1979) concluded that fungi degraded diflubenzuron more rapidly than bacteria.

Diflubenzuron is essentially not metabolized by cotton (Bull and Ivie, 1978; Mansager *et al.*, 1979) and other plants (including soy beans, apple, maize, and cabbage; Verloop and Ferrell, 1977). Absorption, translocation, and photodegradation were insignificant when diflubenzuron was applied to cotton foliage (Bull and Ivie, 1978; Mansager *et al.*, 1979). Cultivation of diflubenzuron-treated cotton into soil followed by planting of wheat and collards resulted in minimal residues in these rotational crops (Bull and Ivie, 1978). Cotton planted in [^{14}C]diflubenzuron-treated soil acquired only 3% of the applied ^{14}C after 89 days and this small residue may be due in part to $^{14}CO_2$ fixation and incorporation into plant structural components (Mansager *et al.*, 1979).

Metabolism of Benzoylphenyl Ureas

Although the metabolic fate of diflubenzuron has been adequately documented, the degradation of relatively few analogues has been reported. As previously discussed, the analogous 2,6-dichlorobenzamide (PH-60-38) received considerable attention as possibly the first benzoylphenyl urea candidate for commercial development (Verloop and Ferrell, 1977). In part because of extended persistence in soil, emphasis shifted to diflubenzuron. The metabolic fate of penfluron was studied in boll weevils (Chang and Woods, 1979b) and house flies (Chang and Woods, 1979a). For both insects penfluron was degraded qualitatively similar to diflubenzuron, but at a slower rate. Bull and Ivie (1980) have reported the metabolic fate of *N*-methyl diflubenzuron and several methoxylated derivatives in the boll weevil, but their results identified no metabolites and were restricted to observation of absorption, excretion, and secretion into eggs. Schaefer and Dupras (1979) examined the environmental stability of SIR-8514, including effects of pH, temperature, sunlight, microbes, and plants.

FUTURE OF INSECT GROWTH REGULATORS

Williams of Harvard popularized the concept of the third 'generation of pesticides' in 1967 with the thesis that the resulting compounds would solve numerous insect control problems. The resulting juvenile hormone mimics (juvenoids) and other insect growth regulators fall short of being panaceas, but they have resulted in marketable compounds. Of greater importance, they represent a concept for pesticide development which is still valid. As an example of the chitin synthesis inhibitors, diflubenzuron has proven to be useful in the control of major pests throughout the world. It should be noted that the term IGR does not confer *a priori* that a compound has only beneficial effects. The benzoylphenyl ureas being considered for development are generally broad spectrum, rather persistent compounds. It is thus necessary to weigh the benefits and risks associated with each compound. Hopefully, the future will see the development of more chitin synthesis inhibitors of varying specificity and selectivity.

Two juvenoids are marketed by Zoecon Corp. (methoprene and kinoprene). Because of their very high biological activity, the sale represents a much larger tonnage expressed in terms of classical pesticides. Methoprene is quite selective, and the majority of sales are confined to controlling insects of medical and veterinary importance. Methoprene will not see large volume use on row, field, or orchard crops. However, its uses are likely to expand in certain clearly defined areas. The prospect of other juvenoids being developed in the immediate future is slight unless there is a change in the philosophy of pesticide usage and in registration procedures. The advantages of juvenoids represent also their limitations. Juvenoids are generally quite selective, and there are very few markets which will bear the cost of registration of such selective compounds. Possible exceptions include compounds active on the boll weevil or specific for the Lepidoptera. There are compounds which show potential in this area. However, it is unlikely that even they will be developed unless several conditions are met. These conditions include development of high resistance to pyrethroids and other insecticides leaving a clear void in the market, economic incentives for the development of compounds which are very safe for humans and the environment, and rapid registration of compounds compatible with IPM programmes. Our knowledge of the environmental stability and metabolism of IGRs in target and non-target organisms provides a sound basis for synthesis of such third generation compounds. Although the technology is present, the economic incentives are regrettably absent.

The most exciting aspect of the IGR field is its future. The rate of discovery of new agents with novel sites of action on the insect integumental, endocrine, or other systems will probably increase. Such compounds will, hopefully, possess more properties of the ideal insecticide and novel structures are certain to present challenges to future pesticide chemists.

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Bowers, W. S., Thompson, M. J., and Uebel, E. C. (1965). 'Juvenile and gonadotropic hormone activity of 10,11-epoxyfarnesenic acid methyl ester', *Life Sci.* **4**, 2323–2331.

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