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METABOLISM AND ENVIRONMENTAL FATE OF JUVENTIDS:
A LEGACY'S RESEARCH IN PERSPECTIVE
AND AN ASSESSMENT OF THE FUTURE

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ABSTRACT

Aspects of juvenile metabolism and environmental degradation are reviewed with an emphasis on pathways of possible significance to insect endocrinology and to xenobiotic metabolism in general. The future of juvenoids as pest control agents is examined in light of their environmental stability.

INTRODUCTION

The last decade witnessed numerous attempts to commercially develop several juvenile hormone mimics or juvenoids. Thus, it may now prove useful to reflect on what has been learned regarding the environmental fate and pathways of degradation of such compounds from both basic and applied perspectives. Such studies on the stability of juvenoids under a variety of conditions not only provide a scientific framework upon which to build a registration package leading to commercial use, but they also have applied and basic significance to several fields. In this chapter, some aspects of the evolution of juvenoid structures will be examined along with an overview of the major routes of degradation of several compounds. The investigation of these routes of degradation was interesting in itself because the juvenoids represented new structural types whose metabolism was not well studied in either insects or mammals. This work also has broad significance because juvenoids are models for
the natural insect hormones as well as being models for terpenoid compounds ubiquitous in our environment. Since terpenes are natural components of foods and fragrances as well as artificial additives to cosmetics and a host of other products, it is surprising that their metabolism has been so superficially studied.

This chapter is not designed to be an exhaustive review; rather, it will serve to highlight some of the interesting discoveries of juvenile metabolites as they apply to broader fields. This information will then be interpreted in light of an assessment of the future for juvenile hormone mimics. For more exhaustive literature reviews, the reader should refer to Zemanek and Quinlan (1976, 1980) and Schooley and Quinlan (1979).

**EVOLUTION OF JUVENILE STRUCTURES AND PATHWAYS OF THEIR DEGRADATION**

Even before epoxide hydration (1) and ester hydrolysis (2) were established as the two major pathways of juvenile hormone (JH) degradation, work was underway to develop structures which were more active and stable in the insect and less expensive to manufacture. Several such compounds are illustrated in Figure 1.

In methoprene, the C-methyl substituent is much more resistant to hydrolysis than the epoxide of juvenile hormone. The dimethyl ester is more stable to base than the natural enone ester of JH while the triethyl ester of hydroprene and the tripropyl ester of methoprene are more sterically hindered than the methyl ester of JH (Hartwick et al., 1973). These compounds have also proven to be refractory to hydrolysis in insects.

![Structures of representative juvenoids illustrating major sites susceptible to environmental degradation and metabolism.](image)

**Fig. 1.** Structures of representative juvenoids illustrating major sites susceptible to environmental degradation and metabolism.
The major pathways of degradation of methanesynthetic degradation (1) (Fig. 1); oxidation, peroxidation, reduction, and isomerization of the diene olefins (2,3); water hydrolysis (4); and degradation of the backbone of the molecule (Dietz et al., 1974a, 1975; Scholowy and Opladen, 1976, and included references). The instability of the diene moiety to ultraviolet radiation limits the usefulness of these compounds under conditions where they will be exposed to intense sunlight (Opladen et al., 1975; Hartwick et al., 1970). For most uses of methanoane, however, such exposure is not a problem. The 3-methyl moiety is very susceptible to metabolism in some insect strains resistant to pesticides (Kama et al., 1977). Such potential resistance problems have not yet limited the use of methanoane.

A second approach toward designing a stable, relatively inexpensive structure is illustrated by Stauder's 8,10,12-hexatriene ethers. The epoxide moiety is retained, but the water moiety is replaced by the much more hydrodynamically stable phenoxycyan ether linkage. Major routes of degradation involve hydration or rearrangement of the 1,2-epoxide functionality (1) (Fig. 1), epoxidation of the 2,3-olefin (4), allylic hydroxylation leading in some cases to cleavage of the ether linkage (5), and oxidation of the epoxide substituent primarily in the alpha but also in the terminal beta position (6) (Gill et al., 1972, 1974; Hoffman et al., 1973; Hauen et al., 1974; Lise et al., 1976).

To 10-130, developed by Hoffman Lohme, illustrates how other modifications can substantially increase the stability of a molecule. The epoxide branch on the epoxide functionality (7) sacrifices activity on Digaer for increased activity on Lygodesma and several other insects (Curvilles, 1976). Interestingly, the increase in hydrodynamically about the epoxide increases its stability to acid-catalyzed hydrolysis (Kamptner et al., 1974) and this concept was extended to several other compounds (Huyen and Hamock, 1979). Elimination of the 2,3-olefin leads to some loss in activity but also leads to a much more stable compound since the terpenoid chain is no longer susceptible to allylic hydroxylation. The major route of metabolism still involves epoxide hydration (2) and reduction on the phenoxycyan side chain (8) as in 8,10,12-hexatriene (Hauen et al., 1973; Dor et al., 1978). The phenoxycyan side chain of both B 2046B and B 10-130 is susceptible to rapid oxidation. The epoxide moiety is quite liable to hydrolysis under acidic conditions, but it is much more stable than has been widely assumed. Resistant strains of house flies have not been shown to have increased epoxide hydrolysis activity (Ravich et al., 1977; Yen and Tefre, 1978), and epoxide hydration does not appear to be the major route of 3B degradation in any insects. Thus, knowledge of the major mechanisms of hydration of the epoxide moiety
In model ecosystems and in target insects may allow the structure to be metabolized while retaining insecticidal activity. In general, these terpenoid phenox ethers are more stable than the dieneate juvenoids, but they are unstable under conditions where epoxide hydration can occur.

A continuation of this evolution in structure is illustrated by MV 678 (Fig. 1), where the terpenoid phenox ether linkage is eliminated (Schwartz et al., 1974). This modification might lead to enhanced stability since oxidation alpha to a heterocyclic is common in biological systems; however, it also opens a second site for benzylic oxidation. In contrast, the hexyryl substitution on the phenyl moiety may sterically hinder benzylic oxidation, in some cases.

The decision to develop methoprene, hydroprene, R 20458 and Bo 10-1108 were all made at a time when society seemed to desire very nonpersistent compounds. Although each of the above compounds is more stable than the parent IM's, they are very unstable when compared with many classical pesticides. Since juvenoids act only at certain times during insect development, their biostability must be consistent with their mechanism of action. Thus, the above compounds were only suitable for use under fairly restrictive sets of circumstances. Apparently, the disadvantages of juvenoids including their inability to rapidly kill the larval stages of many destructive insects outweighed the advantages offered by the above compounds for many applications.

The concept has recently evolved that persistence need not be necessarily equated with biostability and the resulting bioaccumulation and accumulation in a food chain. Juvenoids are needed which are persistent enough under a variety of field conditions to effectively control pest insects. They need to be refractory to rapid metabolism in certain pest insect systems that have developed very high capacities to degrade foreign compounds, while still having several sites susceptible to metabolism in a variety of organisms. These and other constraints on future juvenoid development will be discussed later in this chapter and by Mein and others elsewhere in this volume.

CONTRIBUTIONS OF JUVENOID RESEARCH TO THE SCIENCE OF METABOLIC CONVERSION

The study of xenobiotic metabolism certainly has applied significance; mainly in gaining approval to market a compound and subsequently in clearing information which might lead to the production of more useful derivatives by increasing or decreasing their biostability, changing their distributivity in various pools within an organism or in an ecosystem, or even designing intrinsically more active compounds. Yet, xenobiotic metabolism is also a basic science. An understanding of how living creatures cope with foreign as well as intrinsic compounds is fundamental to an understanding of how
they exist in ecosystems filled with both natural and man-made polisons.

The first studies on the metabolism and environmental fate of juvenile
came at a time when the techniques of metabolism chemistry were rapidly
evolving. The use of radiotracers, functionality selective degradatives,
and thin-layer chromatography was supplemented by high-performance liquid
chromatography and mass-spectrometry interfaced with gas liquid
chromatography. These techniques allowed the separation of metabolites
of very similar chemical properties and facilitated structural assignments
using very small amounts of samples. The studies on JH and juveind metabolism
in insect organs held in vitro and in isolated hepatocytes represent attempts
to bridge the gap between metabolism studies performed in well defined
cell-free systems and the more complex in vivo studies. It is safe to
predict that such techniques will continue to evolve and find increasing
application in metabolic studies involving a variety of xenobiotics.

The study of juvenile metabolism has uncovered several interesting
metabolic pathways in both target and nontarget organisms. In some cases,
the study of juvenile metabolism has provided information on the metabol-
ism of the natural hormone in insects. Even before the metabolism of
the natural JH's was investigated, the high biological activity of certain
juvenoids indicated that oxygen hydroxylases and esterases were probably
involved in JH metabolism. The report that the cdl was a major metabolite
of the juvenile B 20488 in locust was coincident with a report that JH
carb was an important JH metabolite in several insects (Gill et al., 1972;
Slade and Edlin, 1972). An unexpected metabolic pathway of methoprene
in the housefly involved incorporation of the active 22 isomer to the
much less active 22 isomer (Quinlan et al., 1975). The involvement of a
third group in this reaction is expected since bioisohelpal is used to
isomerize dienone acids during their manufacture by a reversible Michaeli-
like addition (Gobbin, 1970). The reaction was verified in vivo in
resistant and nonresistant house fly strains and glutathiones was shown to
be capable of catalyzing both the forward and the reverse reaction as well
as capable of very slowly conjugating with methoprene and JH, but an
adequate (in vivo mode) was not demonstrated (Nimark et al., 1977,
unpublished). This observation certainly deserves further exploration,
especially as it might apply to the metabolism of the natural hormones.

An important point in the investigation of the metabolism of bio-
degradable pesticides was raised by Quinlan et al. (1974b). When a
potential of other xenobiotic was administered to a nontarget organism,
rapid and quantitative excretion of the radiolabel was considered an
indication of biodegradability. The opposite situation was usually taken
as an indication of undesirable persistence. In mammals, methoprene
was found to be rapidly excreted as a variety of metabolites, including\n\[\text{CO}_2\], indicating that it was rapidly metabolized, but a surprisingly large
proportion of the dose remained, apparently tissue bound. It was then
demonstrated that methoprene was metabolized to $^{14}$C-extracts which was
then incorporated into natural metabolites. Thus, the lack of oral
carcinogenicity of methoprene indicated neither persistence nor the formation
of reactive metabolites but, in contrast, extreme biodegradability. As
pest control agents are patterned more closely after natural products,
this phenomenon is likely to be commonly observed.

Studies on the metabolites of R 20438 led to several interesting
problems. Metabolism by call-free liver homogenates from several species
qualitatively demonstrated the major routes of metabolism observed in vivo
with one major exception. Then-le were seen in vivo metabolites while
significant other cleavage was not detected in vitro (Hoffman et al., 1973;
Gill et al., 1972, 1974; Hammock et al., 1974a). Thus, an in vitro model
for the major route of metabolism of these compounds has not been
established.

Hoffman et al. (1973) reported metabolites of R 20438 in which the
aryl substituent on the phenyl ring was replaced by a p-hydroxy substituent.
These workers speculated that the metabolites might be produced from a
Bayer-Inglis-like oxidation of the arachidonyl moiety. Once again, this
intra-ring oxidation was not duplicated in vitro. These two observa-
tions are certainly deserving of further work since they might illustrate
new pathways of metabolism which could be applicable to a wide
range of xenobiotics.

Several interesting pathways of environmental degradation involve
either the 6,7-epoxide or an epoxide formed in the 2,3-position. Hydrolysis
of the 6,7-epoxide (C) (Fig. 2; 10,11 epoxide of JN) to the corresponding
diol (B) was shown quite early to occur under a variety of conditions. It
was logical to assume that epoxidation and subsequent hydrolysis of the
2,3-epoxyster (C) would lead to a 1,3,4,5,7-pentaoxadihydroxyester (B) (Fig. 2) (Gill et al.,
1972; Austen and Biddiford, 1973). Instead, it was found that hydrolysis of
the diol ester (C) catalyzed by either enzymes or acid or oxidation of the
2,3-epoxyster of the 6,7-diol led to a diol-epoxide (B) intermediate which
rapidly cyclized at or below physiological pH to a variety of products.
Two tetracyclic products (D) were found to be the major products, and
under some circumstances, the only products (Hammock et al., 1974a,b).
Gill et al., 1974). These cyclized products represent minor in vivo and
in vitro metabolites of R 20438. Analogous reactions have been demonstrated
with JN (Hammock et al., 1975) and there is good evidence that the resulting
products are JN metabolites in the house fly (Hammock et al., 1977; Yen
and Terrell, 1980). Impregnated compounds commonly exhibit such 1,5-
unsaturation, and it is likely that many of these compounds are metabo-
lized to analogous cyclic products. The intermediate diol-epoxide is
quite reactive, but with R 20438 and related compounds it, fortunately,
Fig. 2. Metabolism of the epoxide and olefinic functionalities in terephthalic molecules.

Another very interesting observation regarding the 6,7-epoxide moiety of R 20458 was also made by Huffman et al. (1973). When R 20458 was orally administered to rats, several metabolites with the 6,7-olefin were isolated implying epoxide reduction. Since Gill et al. (1972, 1974) failed to find such compounds following intraperitoneal administration of R 20458 to rats and mice, Huffman et al. (1973) speculated that such metabolites might have arisen from olefinic impurities in the R 20458 administered to the rats. This dilemma was explained when the rate of R 20458 in vivo was examined (Ivey et al., 1976; Ivey, 1979). These workers found that under anaerobic conditions the terephthalic acids were capable of selectively reducing R 20458 and several other epoxides to their corresponding olefins (P). Such reactions would be anticipated to be much more important following oral administration than following intraperitoneal administration. Analogous reactions have been found to be catalyzed by the gut contents of a variety of mammals, including man. Since the 6,7-olefin of R 20458 (and the analogous olefin of aequanil and a variety of terephthalic) is readily oxidized, the reaction should effectively be reversible in vivo. The metabolism
of epoxidation compounds is being extensively studied in relation to mutagenicity and carcinogenicity, so it is surprising that this apparent epoxide reduction has not been further investigated.

Epoxide hydration to the diol (8) (Fig. 7) accounts for a major proportion of both in vivo and in vitro metabolites in mammals; however, when the reaction was studied in more detail, it was found to be largely catalyzed by a soluble enzyme apparently located in both the cytosolic and mitochondrial subcellular fractions of mouse liver homogenates (Gill et al., 1972, 1974). This observation was surprising because it had been widely assumed that epoxide hydrolases were exclusively microsomal bound (Quach, 1977). Subsequently, a soluble epoxide hydrolase has been found to occur in the cytosol and mitochondrial lumen in a variety of tissues from numerous vertebrates (Gill and Ranney, unpublished). In contrast to earlier assumptions, this enzyme appears to hydrate a variety of structurally dissimilar epoxides including known mutagens and suspect carcinogens (Fox and Ranney, 1980; Ranney et al., 1982a,b).

This discovery and the observations mentioned above aptly illustrate how the investigation of the metabolic fate of juvenile doses has expanded our understanding of xenobiotic metabolism in general.

FUTURE OF JUVENOIDS: CONSTRAINTS ON DEVELOPMENT

The hope and the reality, Williams (1967) predicted a bright future for third generation pesticides due to their many advantages over the more classical pesticides. The effect agent in developing juvenile doses of the third generation pesticides has certainly not been wasted since the information gained has greatly expanded our knowledge in a variety of fields. Very important to pest management is the evolution in thought leading to a broader definition of the third generation pesticide concept. Initially, a third generation pesticide was defined in terms of a final product acting to disrupt cholinergic mediation in insects. One can conceive of a third generation pesticide in broader terms or as a compound designed to have many of the attributes of an ideal pest management agent whose development has been based on a knowledge of the comparative physiology and biochemistry of target and nontarget organisms. Thus, the third generation concept becomes a dynamic term describing the evolution of a pest management agent rather than simply a mechanism of action (Sparks and Ranney, 1980).

On the other hand, the practical use of juvenile doses has been very limited. Only the diacate juvenile doses have been registered, and of these, metaphost does not appear to be effective in controlling insects on field, row or orchard crops. From an insect pest management standpoint, juvenoids have thus failed to have an important
Impact on the pattern of pesticide usage since most pesticides are used for crop protection. Such large volume markets are obviously the most profitable, and juvenoids have failed to acquire a share of these markets. The capital investment leading to the development of juvenoids has been socially important in expanding basic knowledge and in providing an effective control agent for some medically important insects. However, the small return on this investment makes it of questionable value from an industrial standpoint.

Conditions for future development. Certain social and economic conditions may again make the development of juvenoids and related innovative compounds attractive to future investment. These constraints concern a void in the pesticide market under conditions which the importance of the many advantages of juvenoids will be maximized. This void may be provided by the development of resistance to phosphates, carbamates and other poisons in certain key pests (Shweiky and Saito, 1980). Although juvenoids can clearly become resistant to juvenoids, it is reassuring that there is no good evidence for resistance involving the site of action. The juvenoids to be developed must not be susceptible to high levels of cross-resistance in major pests selected with classical pesticides. Thus, including several resistant strains at the stage in a screening program would be wise (Sparks and Tannock, 1980).

The philosophy of total pest management has attracted scientists for years, and recently there seems to be a growing acceptance of well-designed insect pest management programs. The selectivity of juvenoids among insect orders and even families is much greater than that commonly observed with classical pesticides. This advantage of juvenoids may make them more economically attractive as pest management programs become increasingly common.

During the 1950's and 1960's, there was a trend toward developing compounds that were much less persistent than the organochlorine pesticides due to a concern over the accumulation of pesticides in food chains and the effect which trace levels of dietary pesticides might have on man. Although juvenoids are quite biodegradable, so are a number of classical pesticides. Thus, the persistent organochlorine pesticides generally with very low mammalian toxicity, were often replaced with less persistent organophosphate and carbamate pesticides often with high mammalian toxicity. Protection of the environment and the concern from trace levels of pesticides was, thus, often obtained at the expense of agricultural workers having to use acutely toxic materials. During the last part of this decade there has been increasing concern for the health of the workers employed in the manufacture, distribution and use of pesticides as well as for those inadvertently exposed during cultivation or harvest or due to pesticide drifts. If this trend continues, the extraordinarily low mammalian toxicity of juvenoids may provide an economic incentive
encouraging their development. A number of factors, certainly including resistance, can be anticipated to create potential markets for new pesticides. With increasing concern for compounds which are safe to use and which can be integrated into pest management programs, new juvenile hormones may find application.

Even assuming that the socioeconomic constraints are adequate for the development of a juvenile, these are certain constraints on the compound itself. In addition to efficacy, these constraints are related to the selectivity, cost and persistence of the candidate insecticides. Since many of the advantages of juvenoids are also disadvantages, balances must be obtained. The selectivity of juvenoids is certainly an advantage, but high selectivity more or less than other factors has probably limited their use in the field because the available markets are not large enough to bear the expense of registration. Only a very few individual insects, such as the boll weevil, could support the cost of developing a pesticide. On the other hand, a compound selective for the order Lepidoptera could represent an important pest management tool as well as a sound investment. The effectiveness of juvenoids indicates that they will have a high profit margin. Still, the development of compounds which are less expensive to produce than the earlier juvenoids is attractive.

In this chapter, aspects of the fate of juvenoids in the environment and in nontarget organisms have been examined. It is clear, for many reasons, that more stable juvenoids should be developed under conditions of use. The approach described, in part, by Fungartner et al. (1976) in which an estimate of environmental stability is incorporated quite early into efficacy studies, might be valuable in assessing new compounds. From the numerous studies on the degradation of juvenoids and other xenobiotics, certain functionalities are ubiquitously too unstable to incorporate into a molecule designed for field use. A move toward making a compound stable enough to effect good insect control is not necessarily synonymous with the development of a persistent pesticide. For many applications, stability to light is a critical factor while hydrolytic stability is less important in determining efficacy under field conditions. The presence of one or more hydrolytically unstable moieties will lead to a compound that will not be overly persistent once it has weathered or entered a food chain. A fairly complex parameter to adjust is the stability in target vs. nontarget organisms. Compounds highly refractory to metabolism are not socially acceptable due partly to their tendency to accumulate in a food chain, yet many target organisms are remarkably efficient at degrading xenobiotics. One approach is to develop compounds moderately resistant to rapid biological oxidation while still retaining some sites susceptible to hydrolysis. The juvenoid No 13-5223 discussed by Glueckert and coworkers...
In this volume may approach many of the attributes discussed here. The
data presented in this volume clearly indicate the effectiveness of the
compound, and it is interesting to see if it will also prove to be
economically profitable.

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851