Journal of Chemical Ecology, Vol. 14, No. 10, 1988

ADAPTIVE RELATIONSHIPS OF EPOXIDE HYDROLASE IN HERBIVOROUS ARTHROPODS

CHRISTOPHER A. MULLIN

Department of Entomology
Pesticide Research Laboratory and Graduate Study Center
The Pennsylvania State University
University Park, Pennsylvania 16802

(Received September 15, 1987; accepted March 15, 1988)

Abstract—Epoxide hydrolase catalyzes a simple hydrolysis of reactive cyclic ethers that may otherwise alkylate and impair critical proteins and nucleic acids required for life. Although much less studied than the cytochrome P-450 monooxygenases that produce epoxides, differences in subcellular, tissue, pH, substrate, and inhibitor specificities argue for at least three forms of insect epoxide hydrolase. Increasing numbers of epoxides are being identified as plant allelochemicals, antifeedants, and essential hormones or precursors for herbivorous arthropods, and in many cases an associated alkene to diol pathway of metabolism is found. A role for epoxide hydrolase in arthropod–plant interactions is strongly supported by species comparisons and by age–activity and induction studies. Two major limitations for study in biochemical ecology of epoxide hydrolase are the lack of an effective in vivo inhibitor and a range of commercially available radiolabeled substrates for the enzymes.

Key Words—Epoxide hydrolase, insect, herbivore, plant epoxides, alkene, diol, coadaptation, detoxification, arthropod-plant interactions.

INTRODUCTION

Considerable effort has been invested in determining adaptive roles for detoxification enzymes in plant-feeding insects. The tens of thousands of potentially toxic secondary metabolites in plants, together with the exceptionally high levels of oxidative, hydrolytic, and conjugative xenobiotic metabolizing enzymes in insect herbivores, have stimulated much discussion of an associative role. This paradigm has been more rigorously established by recent study on specific

0098-0331/88/1000-1867$06.00/0

dietary allelochemicals as inducers for and substrates of enzymes that metabolize foreign compounds in the respective herbivore (Ahmad, 1986; Brattsten, 1979; Brattsten and Ahmad, 1986; Dowd et al., 1983; Hodgson, 1985). Most work has concentrated on cytochrome P-450-dependent monooxygenases (EC 1.14.14.1) because of their pivotal role in initiating the concerted enzymatic reactions by which lipophilic, nonnutrient compounds are rendered into water-soluble and more excretable metabolites. Among the reactions catalyzed by these polysubstrate monooxygenases (PSMOs) are π-bond oxygenations forming epoxides. These important reactive intermediates can then be hydrolyzed to diols by epoxide hydrolases (EC 3.3.2.3) or conjugated to glutathione by glutathione transferases (EC 2.5.1.18). The three aforementioned enzyme systems constitute, within arthropods, the major enzymatic pathways by which epoxides are formed and degraded, of which the epoxide hydrolase is the least studied.

Many epoxides are strong electrophiles and can undergo rapid nucleophilic additions during both routine isolation and in vivo with water, sulfhydryl, and amino groups; they also participate in acid-enhanced rearrangements and polymerization. The high chemical reactivity of epoxides is often responsible for their high toxicity, mutagenicity, carcinogenicity, and other biological activities (Casida and Ruzo, 1986; Hutson, 1983). In turn, the presence of the usually less toxic *trans* 1,2-diol in an organism is good evidence that a PSMO-initiated alkene to diol pathway exists (Hsia, 1982–1983). There are presently no useful in vivo inhibitors of epoxide-metabolizing enzymes that can facilitate the isolation of the epoxide intermediate.

Investigators had recognized much earlier the occurrence of *trans*-diol metabolites from some aromatic hydrocarbons and alkenes, but it was not until 1950 that an enzyme responsible for their generation from epoxides was first postulated (Boyland, 1950). A few years later, a search for soil microbes capable of growth on an epoxide of fumaric acid as their sole carbon source led to the discovery that cell-free preparations from both a *Flavobacterium* spp. and the fungus *Aspergillus fumigatus* were able to convert *trans*-L-epoxysuccinic acid (**I**, Figure 1) to *meso*-tartaric acid (Martin and Foster, 1955). Subsequently, Breuer and Knuppen (1961) reported the *trans*-hydration of epoxyestrogens in rat liver slices. In plants, the simultaneous occurrence in ironweed (*Vernonia anthelmintica*) seeds of the appropriate epoxide and diol provided a base for postulating an enzyme that converts *cis*-12,13-epoxyoleic acid (**II**) to *threo*-12,13-dihydroxyoleic acid (Scott et al., 1963). Also, high levels of epoxidized fatty acids together with very active epoxide hydrolases for these substrates were found in spores of plant rusts (Hartmann and Frear, 1963; Tulloch, 1963).

The first demonstration of epoxide hydrolase in insects occurred with a synthetic cyclodiene insecticide. In vivo studies in dieldrin-resistant southern house mosquitoes (*Culex pipiens quinquefasciatus*) (Oonnithan and Miskus,

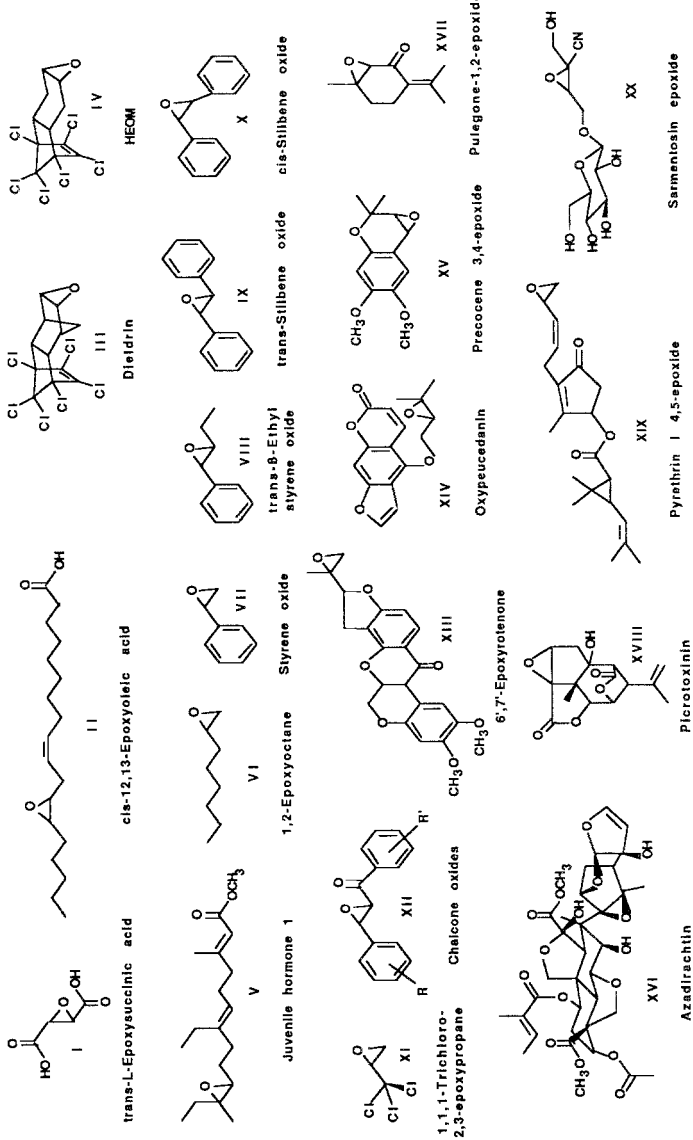

FIG. 1. Some epoxides associated with arthropod–plant interactions.

1964; Tomlin, 1968) had previously established that dieldrin (**III**) was metabolized to a product cochromatographing with aldrin *trans*-diol; similarly, house flies topically treated with chlordene epoxide formed compounds thought to be chlordene glycols (Brooks and Harrison, 1965). The enzymatic nature of this epoxide hydrolase activity towards dieldrin, its analog HEOM (**IV**), and other cyclodiene epoxides was finally verified in vitro in preparations from the house fly, and in rat and pig liver microsomes (Brooks, 1966; Brooks et al., 1970). Epoxide hydrolase has subsequently been found in all living organisms investigated. Comprehensive reviews emphasizing mammalian (Oesch, 1973; Lu and Miwa, 1980; Seidegard and DePierre, 1983; Wixtrom and Hammock, 1985) and insect (Brooks, 1977; Hammock, 1985) epoxide hydrolases are available. The consequence of epoxide hydrolase in herbivorous arthropods is now addressed.

PROPERTIES OF ARTHROPOD EPOXIDE HYDROLASE

Epoxide hydrolase has been purified from only one insect source, that of the southern armyworm, *Spodoptera eridania*, a polyphagous herbivore (Mullin and Wilkinson, 1980a). The larval midgut enzyme has an extraordinarily wide specificity for substrates and hydrolyzes a range of epoxides from simpler monosubstituted compounds to more sterically hindered compounds such as juvenile hormone (**V**) and chlorinated cyclodiene epoxides. Moreover, the activities for the latter substrates were 100–30,000 times lower than activities for 1,2-epoxyoctane (**VI**), styrene oxide (**VII**), and other less-hindered substrates (Mullin and Wilkinson, 1980b). Armyworm epoxide hydrolase, the most active eukaryotic preparation so far reported, was calculated to comprise 3.7% of the total microsomal protein in the armyworm midgut. This indicates an important role for the enzyme in this herbivore, although the low activity for juvenile hormone (JH) and cyclodiene epoxides in comparison to activities in crude preparations suggests that other isozymes are present in armyworm midgut (Mullin and Wilkinson, 1980b).

Multiple Forms. Tissue and subcellular distributions, pH optima, and substrate and inhibitor specificities strongly support the existence of multiple forms of epoxide hydrolase in arthropods. Highest activities towards commonly used substrates occur in the midgut microsomes at alkaline pH. JH epoxide hydrolase appears more widely in fat body (Fox and Massare, 1976; Wing et al., 1981) and other tissues such as the Malpighian tubules, integument (Slade and Wilkinson, 1974), wing imaginal disks (Hammock et al., 1975), silk gland (Wisniewski et al., 1986), and even the corpora allata (Gadot et al., 1987). Also, considerable JH epoxide hydrolase is cytosolic rather than microsomally bound (Yu and Terriere, 1978b; Wisniewski et al., 1986), although this is not always

the case (Hammock et al., 1974, 1975). By contrast, epoxide hydrolases for the cyclodiene HEOM (Slade et al., 1975), styrene oxide, and 1,2-epoxyoctane (Mullin and Wilkinson, 1980a,b) in the southern armyworm are largely microsomal. Recent studies with the substrates *trans*-β-ethylstyrene oxide (**VIII**), *trans*-stilbene oxide (**IX**), and *cis*-stilbene oxide (**X**) (Table I) indicate that considerable activity is cytosolic, particularly for saprophagous compared to herbivorous arthropods. In the laboratory fruit fly, *Drosophila melanogaster*, even styrene oxide, a substrate normally hydrated preferentially in microsomes (Wixtrom and Hammock, 1985, and references therein), is rapidly hydrolyzed in the cytosol (Jansen et al., 1986).

Differences in pH optima also suggest the presence of multiple epoxide hydrolases. In *S. eridania* midgut microsomes, both HEOM hydrolase (Slade et al., 1975) and 1,2-epoxyoctane hydrolase (Mullin and Wilkinson, 1980a) are optimal between pH 8.5 and 9.5 in Tris and glycine buffers, while JH epoxide hydrolase has an optimum of 7.9 (Slade et al., 1975). Activity in cabbage looper, *Trichoplusia ni*, midgut microsomes peaks at pH 7.4 for *trans*-stilbene oxide and pH 8.0 for *cis*-stilbene oxide (Ottea and Hammock, 1986). In other species, there is a similar tendency for hydrolases of *trans*-disubstituted and higher substituted epoxides such as JH to have more acidic pH optima than those for monosubstituted and *cis*-disubstituted substrates (Hammock et al., 1974; Cohen, 1981).

At present there are no effective *in vivo* inhibitors of epoxide hydrolase,

TABLE I. SUBCELLULAR DISTRIBUTION OF EPOXIDE HYDROLASE IN ARTHROPODS

Feeding strategy and Species	Tissue	Relative specific activity (microsomes/cytosol)	
		trans-β-Ethylstyrene oxide	*cis*-Stilbene oxide
Herbivore			
Northern corn rootworm[a]	Midgut	9.5	9.4
Cabbage looper[b]	Midgut	8.2	7.7
Western corn rootworm[a]	Midgut	7.0	6.4
Two-spotted spider mite[c]	Whole body	4.0	2.9
Saprophage			
House fly[d]	Abdomen	1.2[f]	1.0
Laboratory fruit fly[e]	Whole body	0.4[f]	1.0

[a]B.D. Siegfried and C.A. Mullin, unpublished data.
[b]J.A. Ottea and B.D. Hammock (1986).
[c]C.A. Mullin, F. Matsumura, and B.A. Croft (1984).
[d]J.A. Ottea, F.W. Plapp, Jr., and B.D. Hammock (1987b).
[e]J.A. Ottea, L.G. Harshman, and B.D. Hammock (1987a).
[f]Substrate was *trans*-stilbene oxide.

and this greatly limits efforts to understand the toxicological role of the enzyme. The best inhibitors of HEOM and styrene oxide hydrolases in *S. eridania*, *Calliphora erythrocepala*, *Tenebrio molitor*, and *Tribolium castaneum* include 1,1,1-trichloro-2,3-epoxypropane (**XI,** TCEP), phenolic 2,3-epoxypropyl ethers, and some synergists of PSMO (Brooks, 1973, 1977; Slade et al., 1975; Cohen, 1981), and, in addition, sodium picrylsulfonate in *S. eridania* (Mullin and Wilkinson, 1980b). By contrast, JH hydrolase is more sensitive to hormone analogs than to TCEP (Hammock et al., 1974; Slade and Wilkinson, 1973; Yu and Terriere, 1978b). Differential inhibition of *trans*- and *cis*-epoxide hydrolases by TCEP and the equally or more potent chalcone oxides (**XII**) has also provided evidence of multiple isozymes in *Tetranychus urticae* (Mullin et al., 1984), *Delia antiqua*, *Diabrotica barberi*, and *D. virgifera* (Siegfried and Mullin, unpublished data). The chalcone oxides (Mullin and Hammock, 1982) and other flavonoid epoxides such as 6′, 7′-epoxyrotenone (**XIII**; Cova et al., 1986) are strongly inhibitory to mammalian epoxide hydrolases, and in the former case inhibit particularly the *trans*-selective enzymes.

Species Distribution. Epoxide hydrolase has been found in almost 70 insect species (Table 2), and many interspecific variations are apparent. For example, house flies and blow flies (*Calliphora erythrocephala*) readily hydrate the cyclodiene HEOM, but bloodsucking diptera such as the tsetse fly (*Glossina austeni*) and stable fly (*Stomoxys calcitrans*) are deficient in this activity (Brooks, 1977). Most of the early work concentrated on the epoxide hydrolysis of insecticidal chlorinated cyclodienes (Brooks, 1977) and the sesquiterpenoid JHs and their synthetic analogs (Hammock and Quistad, 1976; 1981; Hammock, 1985), and has been exhaustively reviewed. Much less is known about the role of epoxide hydrolase in the metabolism of phytochemical epoxides. Use of appropriate model substrates such as alkylstyrene and stilbene oxides (**VIII, IX, X**) for more complex epoxides in plants has provided new insight into insect–plant relationships. These radiolabeled substrates mimic epoxide metabolites formed in mammals (cf. Scheline, 1978) from phenylpropenoids such as isoeugenol (Mullin, 1985), but they lack phenolic, methoxy, and other functional groups that would result in competing and subsequent reactions that do not allow measurement of specific rates for one enzyme activity. Exceptional levels of epoxide hydrolase acting on these substrates have been found in generalist insect herbivores (Mullin and Wilkinson, 1980a; Mullin and Croft, 1984).

PLANT EPOXIDES VIA THE ALKENE TO DIOL PATHWAY

Plant Epoxides as Allelochemicals. There is now much support to the concept that epoxides contribute greatly to the allelochemical barrier for a prospective arthropod herbivore. Since the characterization of the coumarin oxypeucedanin (**XIV**) in 1933 as the first recognized epoxide in plants, improved

TABLE 2. IN VITRO OCCURRENCE OF EPOXIDE HYDROLASE AMONG INSECTS

Order and Species	Substrate (reference)[a]
Thysanura	
Thermobia domestica	JH (1)
Orthoptera	
Locusta migratoria	JH (9)
Dictyoptera	
Blattella germanica	JH (1), SO (15)
Gromphadorhina portentosa	CE (22), JH (20)
Periplaneta americana	CE (17), JH (1,8,11)
Hemiptera	
Cimex lectularius	CE (5)
Oncopeltus fasciatus	JH (1), SO (15)
Podisus maculiventris	SO (24)
Pyrrhocoris apterus	JH (1)
Rhodnius prolixus	CE (5)
Homoptera	
Aphis nerii	SO (14)
Dactynotus ambrosiae	JH (1)
Macrosiphum euphorbiae	SO (14)
Myzus persicae	SO (15)
Neuroptera	
Chrysopa carnea	SO (15)
Coleoptera	
Acalymma vittata	SO (15)
Altica woodsi	SO (15)
Chauliognathus pennsylvanicus	SO (15)
Coccinella novemnotata	SO (15)
Coleomegilla maculata	SO (15)
Crioceris asparagi	SO (15)
Diabrotica barberi	SO (15)
D. undecimpunctata howardi	SO (15)
D. virgifera virgifera	SO (15)
Epicauta pennsylvanica	SO (15)
Epilachna varivestis	SO (15)
Hippodamia convergens	JH (1), SO (15)
Leptinotarsa decemlineata	JH (1,12), SO (15)
Plagiodera versicolora	SO (15)
Tenebrio molitor	CE (4), JH (1,11)
Tribolium castaneum	SO (6)
Trirhabda virgata	SO (15)
Lepidoptera	
Antheraea pernyi	JH (1), SO (13)
Anticarsia gemmatalis	SO (24)
Argyrotaenia citrana	SO (7)
Choristoneura rosaceana	SO (15)
Galleria mellonella	CE (5), JH (23)

TABLE 2. Continued

Order and Species	Substrate (reference)[a]
Heliothis virescens	SO (24)
H. zea	SO (14,24)
Hyalophora cecropia	JH (1,20)
Hyphantria cunea	SO (15)
Lymantria dispar	SO (14)
Malacosoma americanum	SO (15)
Manduca sexta	CE (5), JH (1,11,20,21)
Philasamia ricini	CE (5)
Pieris brassicae	CE (5)
P. rapae	SO (15)
Samia cynthia	JH (1)
Spodoptera eridania	CE (22), JH (20), SO (16)
S. frugiperda	SO (15,24,26)
Trichoplusia ni	JH (11), SO (18)
Diptera	
Aedes aegypti	CE (5), SO (15)
Anopheles stephensi	CE (5)
Calliphora erythrocephala	CE (4)
Chaoborus americanus	JH (1)
Culex fatigans	CE (5)
Delia antiqua	SO (15)
Drosophila melanogaster	JH (1), SO (2,10,19)
Glossina austeni	CE (4)
Musca domestica	CE (3), JH (1,11,25), SO (15)
Phormia regina	JH (25)
Sarcophaga bullata	JH (11,25)
Stomoxys calcitrans	CE (4)
Hymenoptera	
Apis mellifera	JH (1), SO (26)
Atta texana	JH (1)
Oncophanes americanus	SO (7)
Pediobius foveolatus	SO (15)
Solenopsis invicta	JH (1)

[a]CE = chlorinated cyclodiene epoxide, JH = juvenile hormones and analogs, SO = styrene and stilbene oxides and analogs. References:

1. Ajami and Riddiford, 1973
2. Baars et al., 1979
3. Brooks, 1966
4. Brooks, 1973
5. Brooks, 1979
6. Cohen, 1981
7. Croft and Mullin, 1984
8. Fox and Massare, 1976
9. Gadot et al., 1987
10. Hallstrom and Grafstrom, 1981
11. Hammock et al., 1974
12. Kramer et al., 1977
13. Mullin, 1979
14. Mullin, 1985
15. Mullin and Croft, 1984
16. Mullin and Wilkinson, 1980a
17. Nelson and Matsumura, 1973
18. Ottea and Hammock, 1986
19. Ottea et al., 1987a
20. Slade and Wilkinson, 1974
21. Slade and Zibitt, 1971
22. Slade et al., 1975
23. Wisniewski et al., 1986
24. Yu, 1987
25. Yu and Terriere, 1978a
26. Yu et al., 1984

methods for isolation and identification have led to discovery of hundreds of epoxides in higher plants (Cross, 1960; Dean, 1963; Mullin, 1985). The occurrence of an epoxide substrate for hydration in eukaryotic organisms is often predicted based on presence of the more stable vicinal diol and its respective unsaturated or aromatic hydrocarbon. Plant-derived epoxides such as the allatotoxin precocene 3,4-epoxide (**XV**) (Pratt et al., 1980; Soderlund et al., 1980) and various arene oxides (Brooks, 1979) are so reactive that spontaneous hydrolysis to inactive diols occurs without the intermediacy of epoxide hydrolase.

Surprisingly, alkaloid epoxides such as some pyrrolizidines are sufficiently stable to survive the acid treatments routinely used by alkaloid chemists. The high concentration of acid-stable jacobine with its respective olefin and diol in *Senecio jacobaea* indicates that plants use an enzymatic alkene-to-diol pathway to biosynthesize these alkaloids (Figure 2). Tiger moths that specialize on *S. jacobaea* are known to preferentially sequester the olefin at the expense of the epoxide and diol (Rothschild et al., 1979). The same alkaloids are potent inducers of mammalian epoxide hydrolase (Miranda et al., 1980).

Numerous insecticidal and antifeedant epoxides have been found in plants and may be substrates for the appropriate epoxide hydrolases. Among the plant terpenoids, many insect feeding deterrents including the highly potent azadirachtin (**XVI**) are epoxides (Burnett et al., 1974; Norris 1986; van Beek and de Groot, 1986; Yamasaki and Klocke, 1987), and hydration of the epoxide group can lead to loss of deterrent activity (Kubo and Matsumoto, 1985). Other epoxyterpenoids such as the anticholinesterase monoterpene, pulegone-1,2-epoxide (**XVII**), from *Lippia steochadifolia* (Grundy and Still, 1985), and the sesquiterpene picrotoxinin (**XVIII**) from *Anamirta cocculus* (Miller et al., 1979) are directly insecticidal. Unsaturated monoterpenes of wide occurrence in plants, including limonene (Brattsen, 1983) and α-pinene (White et al., 1979), are readily converted in mammals to *trans*-diols via epoxides; presumably the diols formed from the highly neurotoxic pyrethrins (**XIX**) of *Chrysanthemum ciner-*

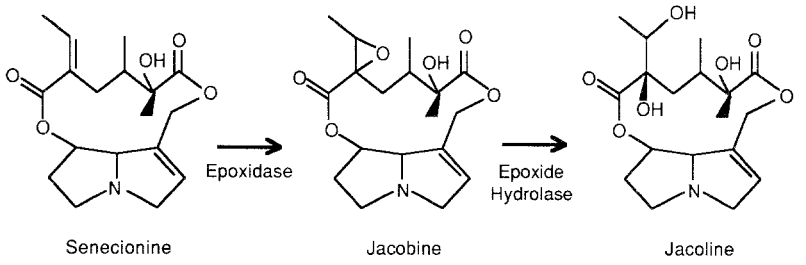

FIG. 2. Possible alkene-to-diol pathway for biosynthesis of pyrrolizidine alkaloids in *Senecio*.

ariaefolium are the result of epoxidation in the alcohol side chain followed by hydrolysis (Casida et al., 1971). Interestingly, epoxidation of synthetic chrysanthemates in the acid side chain results in neuroactive and insecticidal metabolites (Ruzo et al., 1984).

Similarly, epoxy fatty acids make up to 60% of the monomers in cuticular layers of plants and are copolymerized with polyol products of the epoxidase–epoxide hydrolase pathway to give the protective waxy surfaces (Croteau and Kolattukudy, 1974; Holloway and Deas, 1973; Holloway et al., 1981; Kolattukudy, 1985). Insect herbivores may use a *trans*-selective epoxide hydrolase to metabolize these epoxides, as is the case in mammals (Gill and Hammock, 1979).

If increased impact on a herbivore occurs via the more labile epoxide of an unsaturated plant allelochemical, it may be critical that epoxidation in the exposed herbivore occurs at or near the target site of action for the allelochemical. This should maximize the adaptive benefit of synthesizing the respective alkene in the protected plant since epoxide-to-diol detoxification in other tissues of the plant (Banthorpe and Osborne, 1984) or herbivore will reduce efficacy.

Plant aromatics are metabolized in herbivorous insects via the alkene-to-diol pathway. The allatotoxic chromenes from *Ageratum houstonianum*, precocene I and the more active precocene II, undergo bioactivation to cytotoxic 3,4-epoxides in the corpora allata of susceptible insects, such as the large milkweed bug, and thereby impair juvenile hormone biosynthesis (Bowers and Martinez-Pardo, 1977; Pratt et al., 1980; Feyereisen et al., 1981). These epoxides are reactive alkylators of macromolecules. The inactive diol metabolites formed in the corpora allata or peripheral tissues of the insect are largely formed by spontaneous hydrolysis with water (Soderlund et al., 1980) and not by epoxide hydrolase. The insecticidal isoflavonoid, rotenone, is readily detoxified in cockroaches and house flies to a dihydrodiol metabolite (Fukami et al., 1967, 1969) presumably through an epoxyisopropyl intermediate (**XIII**).

Alkene-to-Diol Pathway in Hormone Regulation. Herbivorous insects require epoxidases to synthesize both molting hormones from dietary phytosterols (Fujimoto et al., 1985) and juvenile hormones from the appropriate precursors (Feyereisen and Farnsworth, 1987, and references therein). Some evidence in the former (Rees, 1985) and much for the latter (Hammock, 1985) implicates epoxide hydrolase in regulation of titers of these epoxides. Thus, midgut microsomes of *Spodoptera littoralis* hydrolyze fucosterol-24,28-epoxide to stigmast-5-en-3β,24,28-triol which interrupts the dealkylation sequence and cannot satisfy the sterol requirement of the insect (Figure 3).

ROLE IN HERBIVORY

Evaluating the adaptive role of an enzyme in herbivore toxicology requires its dissimilar expression among species, action on potentially toxic substrates,

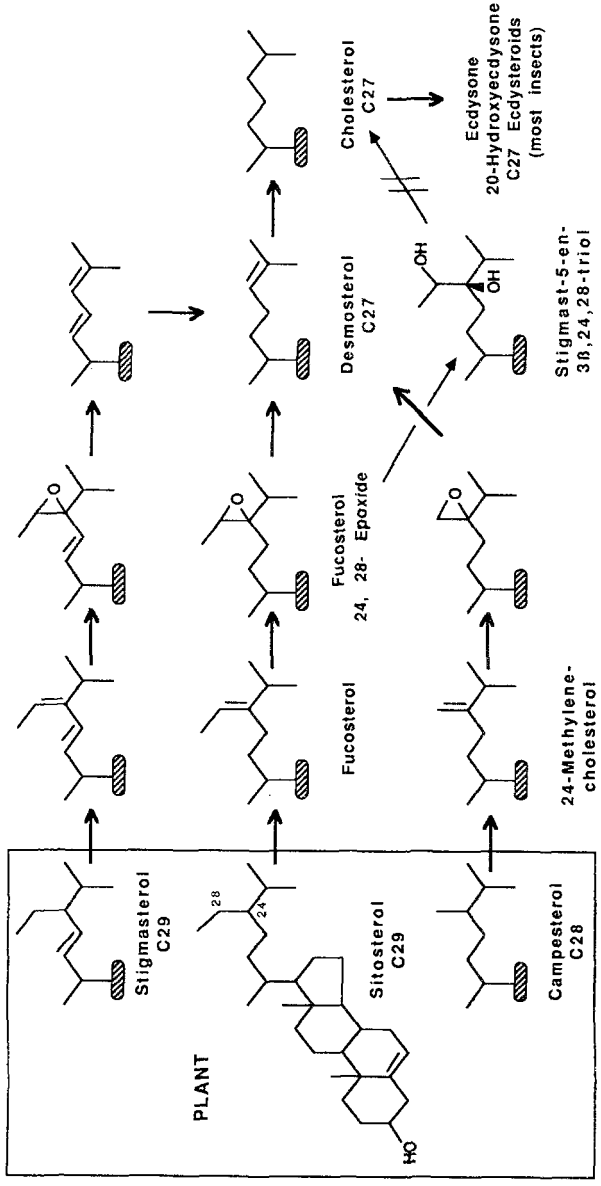

FIG. 3. General scheme for phytosterol dealkylation in herbivorous insects.

and ease of measurement under conditions that do not markedly change from species to species. Epoxide hydrolase shares with PSMO and glutathione transferase a wide and varying distribution among insect species and the ability to detoxify phytochemicals. Moreover, rapid and sensitive assays that easily allow measurements in microarthropods are available for epoxide hydrolase (Wixtrom and Hammock, 1985). Based on energetics, epoxide hydrolase should be less costly to maintain in an organism than PSMO or glutathione transferase since its catalytic mechanism is a simple hydrolysis requiring no coenzymes. By contrast, PSMO is the result of a multienzyme–hemoprotein complex that consumes NADPH and oxygen, while glutathione transferase requires a tripeptide as a cosubstrate. Consequently, epoxide hydrolase measurements should facilitate the study of arthropod–plant coadaptations.

Age–Activity Relationships. A clue to the importance of epoxide hydrolase in metabolism of dietary chemicals comes from age–activity relationships. In the southern armyworm, there is a direct association of high epoxide hydrolase activity with periods of high consumption of plant-based diets (Figure 4). Midgut 1,2-epoxyoctane hydrolase increased sevenfold in total activity during the first 60 hr of the late larval instar before undergoing a dramatic decrease in activity after cessation of feeding and preparation for pupation occurs (Mullin and Wilkinson, 1980a). A similar profile has been noted in this (Slade et al., 1976) and other lepidopteran species (Wing et al., 1981) for HEOM and a juvenile hormone analog. The single peak of JH epoxide hydrolase in the last

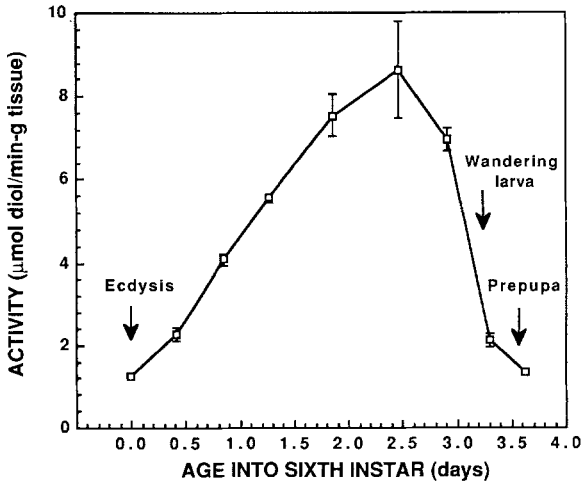

FIG. 4. Age-dependent changes in 1,2-epoxyoctane hydrolase in midgut homogenates from the sixth larval instar of the southern armyworm. Standard error bars for activities shown.

larval instar of Lepidoptera is not as finely synchronized with the biphasic needs for JH degradation as that of JH-specific esterase, which has two distinct peaks of activity. Moreover, epoxide hydrolase activity for JH is an order of magnitude less than the esterase activity (Hammock, 1985), and it is quite likely that the JH epoxide hydrolase is a nonspecific enzyme that can metabolize this and similar sesquiterpenoid substrates that are *trans*- and higher substituted epoxides.

More epoxide hydrolase was found in larvae of both house flies (Yu and Terriere, 1978b) and a braconid ectoparasitoid *Oncophanes americanus* (Croft and Mullin, 1984) than the adults. Larvae process more food than adults and expectedly would encounter more dietary epoxides. However, in Coleoptera very high levels of epoxide hydrolase occur in herbivorous adults (Cohen, 1981), particularly those that are generalists (Mullin and Croft, 1984).

Inducibility. Induction studies also support the contention that epoxide hydrolase is involved in arthropod herbivory. Microsomal epoxide hydrolase activity in southern armyworm midguts was enhanced nearly twofold by feeding larvae for one day on 0.1% pentamethylbenzene in an artificial diet (Mullin and Wilkinson, 1980a). Phytochemicals such as indole 3-carbinol and peppermint oil also induce the enzyme (Table 3). Up to a 3.4-fold increase in *trans*- and *cis*-epoxide hydrolase activities ensues in two-spotted spider mites when fed various host plants (e.g., cotton, lettuce) as compared to snapbean (Mullin and Croft, 1983), although host-related inductive effects were not seen in the fall armyworm with styrene oxide as substrate (Yu and Hsu, 1985). Classical PSMO inducers including phenobarbital in both house fly (Yu and Terriere, 1978b) and *Tribolium castaneum* (Cohen, 1982), and polychlorinated biphenyls and β-naphthoflavone in *Drosophila* (Hallstrom and Grafstrom, 1981) all significantly induce insect epoxide hydrolases. While epoxide hydrolase is, overall, considerably less inducible than PSMO (Brattsten, 1979; Yu, 1986), this may be related to the higher cost of maintaining the multienzyme–coenzyme complex

TABLE 3. INDUCTION OF MICROSOMAL EPOXIDE HYDROLASE IN ARMYWORM MIDGUT

Compound	Percent in diet (wet wt.)	Percent of control activity[a]
Pentamethylbenzene[b]	0.1	196
Phenobarbital[c]	0.2	150
Indole 3-carbinol[c]	0.2	142
Peppermint oil[c]	0.2	134

[a] Styrene oxide as substrate.
[b] Southern armyworm (C.A. Mullin and C.F. Wilkinson, 1980a).
[c] Fall armyworm (S.J. Yu and E.L. Hsu, 1985).

of PSMO in an insect. Thus, the less costly epoxide hydrolase could be kept at sufficient levels to detoxify plant-derived epoxides without a highly primed induction system.

Relationships with Feeding Strategy. Comparison of epoxide hydrolase between herbivorous and nonherbivorous species of arthropods provides additional evidence of its adaptive role. Hydrolase activity for sytrene oxide is higher in chewing herbivores such as lepidopteran larvae than in a predaceous spined soldier bug or insect species that feed on limited portions of the plant (Table 4). However, this substrate efficiently measures only the microsomal activity and may miss other isozymes more selective for plant-derived epoxides.

Use of *trans*-β-ethylstyrene oxide and *cis*-stilbene oxide as substrates in a screen on epoxide hydrolase in 36 arthropod species (cf. Table 2) has led to discovery of more robust differences between ecological groups. Chewing herbivores, on the average, have 21 times more *trans*-epoxide hydrolase and 10 times the ratio of *trans*- to *cis*-epoxide hydrolase than arthropod predators and parasitoids (Mullin et al., 1982; Mullin and Croft, 1984, 1985; Croft and Mullin, 1984; Mullin, 1985). Moreover, phloem-feeders have, on average, 80 times lower *trans*-epoxide hydrolase than herbivores that consume entire plant parts (Mullin, 1986). Further comparisons with chewing and sucking herbivores were made using species from the same insect family. Among 10 species of leaf-feeding beetles (Chrysomelidae), ratios of *trans*- to *cis*-epoxide hydrolase consistently increased ($r > 0.92$) with either number of plant families or plant genera consumed by the species (Mullin and Croft, 1984; Mullin, 1985). In the Aphididae, the highly polyphagous *Macrosiphum euphorbiae* and *Myzus per-*

TABLE 4. MICROSOMAL ACTIVITIES OF STYRENE EPOXIDE HYDROLASE IN SOME INSECTS

Species	Tissue	Styrene epoxide hydrolase (nmol/min/mg protein)	Feeding strategy
Southern armyworm[a]	Midgut	79	Generalist
Fall armyworm[b]	Midgut	26	Generalist
Antheraea pernyi[a]	Midgut	26	Specialist
Spined soldier bug[b]	Midgut	13	Predator
Honeybee[a]	Midgut	8.6	Nectar, pollen
Laboratory fruit fly[c]	Whole	4.3	Saprophagous
Red flour beetle[d]	Whole	2.7	Seed

[a] C.A. Mullin (1979); C.A. Mullin and C.F. Wilkinson (1980a).
[b] S.J. Yu, F.A. Robinson and J.L. Nation (1984); S.J. Yu and E.L. Hsu (1985); S.J. Yu (1987).
[c] A.J. Baars, M. Jansen and D.D. Breimer (1979).
[d] E. Cohen (1981).

sicae had 5–14 times higher epoxide hydrolase activity than the oleander and milkweed specialist, *Aphis nerii*. These results strongly implicate the *trans*-epoxide hydrolase in the metabolism of plant-derived epoxides and support the view that phloem-feeders such as aphids have less epoxide hydrolase than chewing herbivores, since concentrations of secondary plant epoxides in phloem should be less than those of external plant tissues (Mullin, 1986). Coincidently, the plant allelochemicals that may undergo epoxidation, such as unusual fatty acids, cinnamic acids, chalcones, phenylpropenoids, and terpenoids, tend to have a *trans* geometry or are trisubstituted olefins, whereas constitutive olefins common to both animal and plant tissues, such as fatty acids, tend to have a *cis* configuration (Mullin, 1985). Hence, epoxides resulting from epoxidation of available plant allelochemicals in either the plant or its consumer could explain the presence of a high *trans*-selective epoxide hydrolase in herbivorous species.

Epoxide hydrolase may occasionally be counteradaptive to a herbivore. For example, rat liver microsomes greatly facilitated the release of cyanide from a cyanogenic glycoside, sarmentosin epoxide (**XX**), probably by hydration to an unstable cyanohydrin (Nahrstedt et al., 1982). Other diols such as the diterpenoid antifeedant ajugarin III (van Beek and de Groot, 1986) and the synthetic aldrin *trans*-diol (Brooks, 1977; Singh and Singh, 1984) are sufficiently lipophilic to exert near or equal biological activity as the epoxide. However, the net result of increasing water solubility and excretability of an alkene makes the diol conversion a distinct detoxification pathway.

The maintenance of a tightly coupled epoxidase–epoxide hydrolase complex will generally have beneficial consequences for an arthropod herbivore since harmful epoxides will then be immediately dispatched by hydration. It is fortunate that much of cytochrome P-450 and epoxide hydrolase resides together on the endoplasmic reticulum (Brattsen and Ahmad, 1986). Usually the epoxidase is rate-limiting and produces epoxides much slower than the subsequent hydration to diols. Some epoxides such as dieldrin are refractory to hydration and serve as useful products for epoxidase measurements. Hence, the aldrin-to-aldrin diol pathway is an unusual case where epoxide hydration is rate-limiting. Dieldrin and other cyclodiene epoxides are generally recognized as the insecticidal agents in this pathway (Schroeder et al., 1977; Miyazaki et al., 1979; Singh and Singh, 1984), thus a high epoxidase-to-epoxide hydrolase ratio may be disadvantageous to a herbivore exposed to aldrin. Work with corn rootworms, the two-spotted spider mite, and aphids supports this hypothesis in that increased aldrin epoxidase to *cis*-epoxide hydrolase activity correlates with increased susceptibility to aldrin (Mullin, 1986; Mullin et al., 1984) with the potato aphid being less susceptible than the polyphagous northern corn rootworm. Nevertheless, the higher epoxidase and epoxide hydrolase activities of chewing herbivores than phloem-sucking aphids should benefit the former group

since their encounter with olefinic and epoxide phytochemicals is expectedly greater.

ROLE IN CHEMICAL CONTROL OF HERBIVOROUS PESTS

The bulk of insecticides used today lack an epoxide or an unsaturated grouping that will be readily epoxidized, and these groupings are largely removed from promising new insecticides so that improved biostability will be achieved. Chlorinated hydrocarbons where epoxide metabolites are known, including the cyclodienes (Brooks, 1979), DDT (Gold and Brunk, 1982), and lindane (Fitzloff and Pan, 1984), have received increased worldwide disfavor, and no longer are major use insecticides. Aromatic insecticides that undergo ring oxidation to form diols such as the carbamate carbaryl (Kuhr and Dorough, 1976) may do so by spontaneous hydrolysis of the intermediate epoxide, and not necessarily through an epoxide hydrolase. Thus epoxide hydrolase, by design, has a modest role in detoxification of presently used synthetic pesticides. Strategies for overcoming the resistances by arthropods to most available synthetics should not be based on this enzyme. Levels of epoxide hydrolase similar to those of susceptible strains were found in organophosphate-, carbamate-, DDT-, and methoprene-resistant strains of house fly (Hammock et al., 1977; Yu and Terriere, 1978b; Ottea et al., 1987b), organophosphate-resistant two-spotted spider mite (Mullin et al., 1982), carbaryl-resistant fall armyworm (McCord and Yu, 1987), and DDT- and organophosphate-resistant strains of *Drosophila* (Baars et al., 1979; Hallstrom and Grafstrom, 1981). For the latter case, styrene oxide was used as the substrate, whereas in other strains of *Drosophila* with *trans*- and *cis*-stilbene oxides more heterogeneity in epoxide hydrolase was observed (Ottea et al., 1987a). Similar modest increases in epoxide hydrolase were seen in a pyrethroid-, DDT-, and organophosphate-resistant predatory mite, *Amblyseius fallacis* (Mullin et al., 1982), although resistance to juvenoids in the red flour beetle has been associated with a 4.4-fold increase in styrene epoxide hydrolase (Cohen, 1981). Nevertheless, a new insecticide that is detoxified via the epoxide hydrolase should have little cross-resistance in pests that maintain metabolic resistances to other conventional pesticides. Indeed, house flies with multiple resistance to commercial alkoxide juvenoids, such as methoprene, remain susceptible to an epoxide-containing juvenoid (Sparks and Hammock, 1983).

That elevated epoxide hydrolase has not been heavily selected by commercial insecticides, yet is important to a herbivorous pest for detoxification of dietary epoxides, suggests this enzyme as a useful target for future pest control agents. Chalcone oxides and other inhibitors of epoxide hydrolase should synergize the activities of many plant defensive chemicals against herbivorous pests

and have little impact on entomophagous species, since the latter encounter and presumably need to detoxify fewer plants epoxides (Mullin and Croft, 1985).

Chalcones are regarded as universal flavonoid precursors in plants and might serve as endogenous synergists of other plant defenses if oxidized to inhibitors of herbivore epoxide hydrolase. Alternatively, they can direct the synthesis of in vivo inhibitors of epoxide hydrolase that might control populations of herbivorous pests. Additional use of these inhibitors may come through disruption of pheromone perception and deactivation in increasing numbers of herbivore species where major components are epoxides (Ahmad et al., 1986; Bell and Meinwald, 1986; Prestwich and Blomquist, 1987). These studies should give insight into how herbivores successfully coadapt with an epoxide-laden environment.

Acknowledgments—The support of the USDA (82-CRSR-2-2057), NSF (BSR-8306008), Pennsylvania Agricultural Experiment Station (Journal Series No. 7775), and helpful commentary from J. C. Schultz are gratefully recognized.

REFERENCES

AHMAD, S. 1986. Enzymatic adaptations of herbivorous insects and mites to phytochemicals. *J. Chem. Ecol.* 12:533–560.

AHMAD, S., BRATTSTEN, L.B., MULLIN, C.A., and YU, S. J. 1986. Enzymes involved in the metabolism of plant allelochemicals, pp. 73–151, *in* L.B. Brattsten and S. Ahmad (eds.). Molecular Aspects of Insect–Plant Associations, Plenum Press, New York.

AJAMI, A.M., and RIDDIFORD, L.M. 1973. Comparative metabolism of the *Cecropia* juvenile hormone. *J. Insect Physiol.* 19:635–645.

BAARS, A.J., JANSEN, M., and BREIMER, D.D. 1979. Xenobiotic-metabolizing enzymes in *Drosophila melanogaster.* Activities of epoxide hydratase and glutathione *S*-transferase compared with similar activities in rat liver. *Mutat. Res.* 62:279–291.

BANTHORPE, D.V., and OSBORNE, M.J. 1984. Terpene epoxidases and epoxide hydratases from cultures of *Jasminum officinale. Phytochemistry* 23:905–907.

BELL, T.W., and MEINWALD, J. 1986. Pheromones of two arctiid moths (*Creatonotos transiens* and *C. gangis*): Chiral components from both sexes and achiral female components. *J. Chem. Ecol.* 12:385–409.

BOWERS, W.S., and MARTINEZ-PARDO, R. 1977. Antiallatotropins: Inhibition of corpus allatum development. *Science* 197:1369–1371.

BOYLAND, E. 1950. The biological significance of metabolism of polycyclic compounds. *Biochem. Soc. Symp.* 5:40–54.

BRATTSTEN, L.B. 1979. Ecological significance of mixed-function oxidations. *Drug Metab. Rev.* 10:35–58.

BRATTSTEN, L.B. 1983. Cytochrome P-450 involvement in the interactions between plant terpenes and insect herbivores, pp. 173–195, *in* P.A. Hedin (ed.). Plant Resistance to Insects, Symp. Ser. No. 208. American Chemical Society, Washington, D.C.

BRATTSTEN, L.B., and AHMAD, S. (eds.) 1986. Molecular Aspects of Insect–Plant Associations. Plenum Press, New York. 346 pp.

BREUER, H., and KNUPPEN, R. 1961. The formation and hydrolysis of 16α, 17α-epoxyoestratriene-3-ol by the rat liver. *Biochim. Biophys. Acta* 49:620–621.

BROOKS, G.T. 1966. Progress in metabolic studies of the cyclodiene insecticides and its relevance to structure-activity correlations. *World Rev. Pest Control* 5:62–84.

BROOKS, G.T. 1973. Insect epoxide hydrase inhibition by juvenile hormone analogues and metabolic inhibitors. *Nature* 245:382–384.

BROOKS, G.T. 1977. Epoxide hydratase as a modifier of biotransformation and biological activity. *Gen. Pharmacol.* 8:221–226.

BROOKS, G.T. 1979. The metabolism of xenobiotics in insects, pp. 151–214, *in* J.W. Bridges and L.F. Chasseaud (eds.). Progress in Drug Metabolism, Vol. 3. John Wiley, New York.

BROOKS, G.T., and HARRISON, A. 1965. Structure-activity relationships among insecticidal compounds derived from chlordene. *Nature* 205:1031–1032.

BROOKS, G.T., HARRISON, A., and LEWIS, S.E. 1970. Cyclodiene epoxide ring hydration by microsomes from mammalian liver and houseflies. *Biochem. Pharmacol.* 19:225–273.

BURNETT, W.C., JR., JONES, S.B., JR., MABRY, T.J., and PADOLINA, W.G. 1974. Sesquiterpene lactones—insect feeding deterrents in *Vernonia*. *Biochem. Syst. Ecol.* 2:25–29.

CASIDA, J.E., and RUZO, L.O. 1986. Reactive intermediates in pesticide metabolism: Peracid oxidations as possible biomimetic models. *Xenobiotica* 16:1003–1015.

CASIDA, J.E., KIMMEL, E.C., ELLIOTT, M., and JANES, N.F. 1971. Oxidative metabolism of pyrethrins in mammals. *Nature* 230:326–327.

COHEN, E. 1981. Epoxide hydrase activity in the flour beetle *Tribolium castaneum* (Coleoptera, Tenebrionidae). *Comp. Biochem. Physiol.* 69B:29–34.

COHEN, E. 1982. Studies on several microsomal enzymes in two strains of *Tribolium casteaneum* (Tenebrionidae, Coleoptera). *Comp. Biochem. Physiol.* 71C: 123–126.

COVA, D., ARNOLDI, A., COLOMBO, R., and ROSSINI, L. 1986. Stereochemical considerations on the inhibition of hepatic epoxide hydrolase by some pesticides and their epoxides. *Toxicol. Lett.* 30:273–278.

CROFT, B.A., and MULLIN, C.A. 1984. Comparison of detoxification enzyme systems in *Argyrotaenia citrana* (Lepidoptera: Tortricidae) and the ectoparasite, *Oncophanes americanus* (Hymenoptera: Braconidae). *Environ. Entomol.* 13:1330–1335.

CROSS, A.D. 1960. The chemistry of naturally occurring 1,2-epoxides *Qt. Rev. Chem. Soc. London* 14:317–335.

CROTEAU, R., and KOLATTUKUDY, P.E. 1974. Direct evidence for the involvement of epoxide intermediates in the biosynthesis of the C18 family of cutin acids. *Arch. Biochem. Biophys.* 162:471–480.

DEAN, F.M. 1963. Naturally Occurring Oxygen Ring Compounds. Butterworths, London.

DOWD, P.F., SMITH, C.M., and SPARKS, T.C. 1983. Detoxification of plant toxins by insects. *Insect Biochem.* 13:453–468.

FEYEREISEN, R., and FARNSWORTH, D.E. 1987. Precursor supply for insect juvenile hormone III biosynthesis in a cockroach. *J. Biol. Chem.* 262:2676–2681.

FEYEREISEN, R., JOHNSON, G., KOENER, J., STAY, B., and TOBE, S.S. 1981. Precocenes as proallatocidins in adult female *Diploptera punctata*: A functional and ultrastructural study. *J. Insect Physiol.* 27:855–868.

FITZLOFF, J.F., and PAN, J.C. 1984. Epoxidation of the lindane metabolite, β-PCCH, by human and rat liver microsomes. *Xenobiotica* 14:599–604.

FOX, P.M., and MASSARE, J.S. 1976. Aspects of juvenile hormone metabolism in *Periplaneta americana* (L.). *Comp. Biochem. Physiol.* 53B:195–200.

FUJIMOTO, Y., MORISAKI, M., and IKEKAWA, N. 1985. Enzymatic dealkylation of phytosterols in insects. *Methods Enzymol.* 111:346–352.

FUKAMI, J.-I., YAMAMOTO, I., and CASIDA, J.E. 1967. Metabolism of rotenone in vitro by tissue homogenates from mammals and insects. *Science* 155:713–716.

FUKAMI, J.-L., SHISHIDO, T., FUKUNAGA, K., and CASIDA, J.E. 1969. Oxidative metabolism of rotenone in mammals, fish, and insects and its relation to selective toxicity. *J. Agric. Food Chem.* 17:1217–1226.

GADOT, M., GOLDMAN, A. COJOCARU, M., and APPLEBAUM, S.W. 1987. The intrinsic synthesis of juvenile hormone-III diol by locust corpora allata in vitro. *Mol. Cell. Endocrinol.* 49:99–107.

GILL, S.S., and HAMMOCK, B.D. 1979. Hydration of *cis*- and *trans*-epoxymethyl stearates by the cytosolic epoxide hydrase of mouse liver. *Biochem. Biophys. Res. Commun.* 89:965–971.

GOLD, B., and BRUNK, G. 1982. Metabolism of 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane and 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane in the mouse. *Chem.-Biol. Interact.* 41:327–339.

GRUNDY, D.L., and STILL, C.C. 1985. Inhibition of acetylcholinesterases by pulegone-1,2-epoxide. *Pestic. Biochem. Physiol.* 23:383–388.

HALLSTROM, I., and GRAFSTROM, R. 1981. The metabolism of drugs and carcinogens in isolated subcellular fractions of *Drosophila melanogaster*. II. Enzyme induction and metabolism of benzo[α]pyrene. *Chem.-Biol. Interact.* 34:145–159.

HAMMOCK, B.D. 1985. Regulation of juvenile hormone titer: Degradation, pp. 431–472, *in* G.A. Kerkut and L.I. Gilbert (eds.). Comprehensive Insect Physiology, Biochemistry and Pharmacology, Vol. 7, Pergamon Press, Oxford.

HAMMOCK, B.D., and QUISTAD, G.B. 1976. The degradative metabolism of juvenoids by insects, pp. 374–393, in L.I. Gilbert (ed.). The Juvenile Hormones. Plenum Press, New York.

HAMMOCK, B.D., and QUISTAD, G.B. 1981. Metabolism and mode of action of juvenile hormone, juvenoids, and other insect growth regulators, pp. 1–83, *in* D.H. Hutson and T.R. Roberts (eds.). Progress in Pesticide Biochemistry, Vol. 1. John Wiley, New York.

HAMMOCK, B.D., GILL, S.S., and CASIDA, J.E. 1974. Insect metabolism of a phenyl epoxygeranyl ether juvenoid and related compounds. *Pestic. Biochem. Physiol.* 4:393–406.

HAMMOCK, B.D., NOWOCK, J. GOODMAN, W., STAMOUDIS, V., and GILBERT, L.I. 1975. The influence of hemolymph-binding protein on juvenile hormone stability and distribution in *Manduca sexta* fat body and imaginal discs in vitro. *Mol. Cell. Endocrinol.* 3:167–184.

HAMMOCK, B.D., MUMBY, S.M., and LEE, P.W. 1977. Mechanisms of resistance to the juvenoid methoprene in the house fly *Musca domestica* L. *Pestic. Biochem. Physiol.* 7:261–272.

HARTMANN, G.R., and FREAR, D.S. 1963. Enzymatic hydration of *cis*-9,10-epoxyoctadecanoic acid by cell-free extracts of germinating flax rust uredospores. *Biochem. Biophys. Res. Commun.* 10:366–372.

HODGSON, E. 1985. Microsomal mono-oxygenases, pp. 225–321, *in* G.A. Kerkut and L.I. Gilbert (eds.). Comprehensive Insect Physiology, Biochemistry and Pharmacology, Vol. 11. Pergamon Press, Oxford.

HOLLOWAY, P.J., and DEAS, A.H.B. 1973. Epoxyoctadecanoic acids in plant cutins and suberins. *Phytochemistry* 12:1721–1735.

HOLLOWAY, P.J., BROWN, G.A., and WATTENDORFF, J. 1981. Ultrahistochemical detection of epoxides in plant cuticular membranes. *J. Exp. Bot.* 32:1051–1066.

HSIA, M.T.S. 1982–1983. Toxicological significance of dihydrodiol metabolites. *J. Toxicol.-Clin. Toxicol.* 19:737–758.

HUTSON, D.H. 1983. Bioactivation involving chemically reactive oxygenated carbon, pp. 263–274, *in* S. Matsunaka, D.H. Hutson, and S.D. Murphy (eds.). Pesticide Chemistry: Human Welfare and the Environment, Vol. 3 Pergamon Press, Oxford.

JANSEN, M., BAARS, A.J., and BREIMER, D.D. 1986. Microsomal and cytosolic epoxide hydrolase in *Drosophila melanogaster*. *Biochem. Pharmacol.* 35:2229–2232.

KOLATTUKUDY, P.E. 1985. Enzymatic penetration of the plant cuticle by fungal pathogens. *Annu. Rev. Phytopathol.* 23:223–250.

KRAMER, S.J., WIETEN, M., and DEKORT, C.A.D. 1977. Metabolism of juvenile hormone in the Colorado potato beetle, *Leptinotarsa decemlineata*. *Insect Biochem.* 7:231–236.

KUBO, I., and MATSUMOTO, T. 1985. Potent insect antifeedants from the African medicinal plant *Bersama abyssinica*, pp. 183–200, *in* P.A. Hedin (ed.). Bioregulators for Pest Control, Symp. Ser. No. 276. American Chemical Society, Washington, D.C.

KUHR, R.J., and DOROUGH, H.W. 1976. Carbamate Insecticides: Chemistry, Biochemistry and Toxicology. CRC Press, Cleveland, 301 pp.

LU, A.Y.H., and MIWA, G.T. 1980. Molecular properties and biological functions of microsomal epoxide hydrase. *Annu. Rev. Pharmacol. Toxicol.* 20:513–531.

MARTIN, W.R., and FOSTER, J.W. 1955. Production of *trans*-L-epoxysuccinic acid by fungi and its microbiological conversion to *meso*-tartartic acid. *J. Bacteriol.* 70:405–414.

MCCORD, E., JR., and YU, S.J. 1987. The mechanisms of carbaryl resistance in the fall armyworm, *Spodoptera frugiperda* (J.E. Smith). *Pestic. Biochem. Physiol.* 27:114–122.

MILLER, T.A., MAYNARD, M., and KENNEDY, J.M. 1979. Structure and insecticidal activity of picrotoxinin analogs. *Pestic. Biochem. Physiol.* 10:128–136.

MIRANDA, C.L., CHEEKE, P.R., and BUHLER, D.R. 1980. Effect of pyrrolizidine alkaloids from tansy ragwort (*Senecio jacobaea*) on hepatic drug metabolizing enzymes in male rats. *Biochem. Pharmacol.* 29:2645–2649.

MIYAZAKI, A., SAKAI, M., and MARUMO, S. 1979. Comparative metabolism of enantiomers of chlordene and chlordene epoxide in German cockroaches, in relation to their remarkably different insecticidal activity. *J. Agric. Food Chem.* 27:1403–1405.

MULLIN, C.A. 1979. Purification and properties of an epoxide hydratase from the midgut of the southern armyworm (*Spodoptera eridania*). PhD thesis. Cornell University, Ithaca, New York, 231 pp.

MULLIN, C.A. 1985. Detoxification enzyme relationships in arthropods of differing feeding strategies, pp. 267–278, *in* P.A. Hedin (ed.). Bioregulators for Pest Control, Symp. Ser. No. 276, American Chemical Society, Washington, D.C.

MULLIN, C.A. 1986. Adaptive divergence of chewing and sucking arthropods to plant allelochemicals, pp. 175–209, *in* L.B. Brattsten and S. Ahmad (eds.). Molecular Aspects of Insect–Plant Associations, Plenum Press, New York.

MULLIN, C.A., and CROFT, B.A. 1983. Host-related alterations of detoxification enzymes in *Tetranychus urticae* (Acari: Tetranychidae). *Environ. Entomol.* 12:1278–1282.

MULLIN, C.A., and CROFT, B.A. 1984. *trans*-Epoxide hydrolase: A key indicator enzyme for herbivory in arthropods. *Experientia* 40:176–178.

MULLIN, C.A., and CROFT, B.A. 1985. An update on development of selective pesticides favoring arthropod natural enemies, pp. 123–150, *in* M.A. Hoy and D.C. Herzog (eds.). Biological Control in Agricultural IPM Systems. Academic Press, New York.

MULLIN, C.A., and HAMMOCK, B.D. 1982. Chalcone oxides—potent selective inhibitors of cytosolic epoxide hydrolase. *Arch. Biochem. Biophys.* 216:423–439.

MULLIN, C.A., and WILKINSON, C.F. 1980a. Purification of an epoxide hydrolase from the midgut of the southern armyworm (*Spodoptera eridania*). *Insect Biochem.* 10:681–691.

MULLIN, C.A., and WILKINSON, C.F. 1980b. Insect epoxide hydrolase: Properties of a purified enzyme from the southern armyworm (*Spodoptera eridania*). *Pestic. Biochem. Physiol.* 14:192–207.

MULLIN, C.A., CROFT, B.A., STRICKLER, K., MATSUMURA, F., and MILLER, J.R. 1982. Detoxification enzyme differences between a herbivorous and predatory mite. *Science* 217:1270–1272.

MULLIN, C.A., MATSUMURA, F., and CROFT, B.A. 1984. Epoxide forming and degrading enzymes in the spider mite, *Tetranychus urticae*. *Comp. Biochem. Physiol.* 79C:85–92.

NAHRSTEDT, A., WALTHER, A., and WRAY, V. 1982. Sarmentosin epoxide, a new cyanogenic compound from *Sedum cepaea*. *Phytochemistry* 21:107–110.

NELSON, J.O., and MATSUMURA, F. 1973. Dieldrin (HEOD) metabolism in cockroaches and house flies. *Arch. Environ. Contam. Toxicol.* 1:224–244.

NORRIS, D.M. 1986. Anti-feeding compounds, pp. 97–146, *in* G. Haug and H. Hoffman (eds.). Sterol Biosynthesis Inhibitors and Anti-feeding Compounds. Springer-Verlag, New York.

OESCH, F. 1973. Mammalian epoxide hydrases: Inducible enzymes catalysing the inactivation of carcinogenic and cytotoxic metabolites derived from aromatic and olefinic compounds. *Xenobiotica* 3:305–340.

OONNITHAN, E.S., and MISKUS, R. 1964. Metabolism of C^{14}-dieldrin by dieldrin-resistant *Culex pipiens quinquefasciatus* mosquitoes. *J. Econ. Entomol.* 57:425–426.

OTTEA, J.A., and HAMMOCK, B.D. 1986. Optimization of assay conditions for epoxide metabolizing enzymes in *Trichoplusia ni*. *Insect Biochem.* 16:319–325.

OTTEA, J.A., HARSHMAN, L.G., and HAMMOCK, B.D. 1987a. Patterns of epoxide metabolism by epoxide hydrolase and glutathione *S*-transferase associated with age and genotype in *Drosophila melanogaster*. *Muta. Res.* 177:247–254.

OTTEA, J.A., PLAPP, F.W., JR., and HAMMOCK, B.D. 1987b. Biochemical and genetic analysis of epoxide metabolizing enzymes in susceptible and resistant house flies, *Musca domestica* L. *Pestic. Biochem. Physiol.* 29:138–145.

PRATT, G.E., JENNINGS, R.C., HAMNETT, A.F., and BROOKS, G.T. 1980. Lethal metabolism of precocene-I to a reactive epoxide by locust corpora allata. *Nature* 284:320–323.

PRESTWICH, G.D., and BLOMQUIST, G.J. (eds.). 1987. Pheromone Biochemistry. Academic Press, New York, 565 pp.

REES, H.H. 1985. Biosynthesis of ecdysone, pp. 249–293, *in* G.A. Kerkut and L.I. Gilbert (eds.). Comprehensive Insect Physiology, Biochemistry and Pharmacology, Vol. 7. Pergamon Press, New York.

ROTHSCHILD, M., APLIN, R.T., COCKRUM, P.A., EDGAR, J.A., FAIRWEATHER, P., and LEES, R. 1979. Pyrrolizidine alkaloids in arctiid moths (Lep.) with a discussion on host plant relationships and the role of these secondary plant substances in the Arctiidae. *Biol. J. Linn. Soc.* 12:305–326.

RUZO, L.O., CASIDA, J.E., and GAMMON, D.W. 1984. Neurophysiological activity and toxicity of pyrethroids derived by addition of methylene, sulfur or oxygen to the chrysanthemate 2-methyl-1-propenyl substituent. *Pestic. Biochem. Physiol.* 21:84–91.

SCHELINE, R.R. 1978. Mammalian Metabolism of Plant Xenobiotics. Academic Press, New York. 502 pp.

SCHROEDER, M.E., SHANKLAND, D.L., and HOLLINGWORTH, R.M. 1977. The effects of dieldrin and isomeric aldrin diols on synaptic transmission in the American cockroach and their relevance to the dieldrin poisoning syndrome. *Pestic. Biochem. Physiol.* 7:403–415.

SCOTT, W.E., KREWSON, C.F., LUDDY, F.E., and RIEMENSCHNEIDER, R.W. 1963. *Vernonia anthelmintica* (L.) Willd. enzyme studies. Conversion of epoxyoleic acid to (+)-*threo*-12,13-dihydroxyoleic acid. *J. Am. Oil Chemists' Soc.* 40:587–589.

SEIDEGARD, J., and DEPIERRE, J.W. 1983. Microsomal epoxide hydrolase. Properties, regulation and function. *Biochim. Biophys. Acta* 695:251–270.

SINGH, G.J.P., and SINGH, B. 1984. Action of dieldrin and *trans*-aldrindiol upon the ultrastructure of the sixth abdominal ganglion of *Periplaneta americana* in relation to their electrophysiological effects. *Pestic. Biochem. Physiol.* 21:102–126.

SLADE, M., and WILKINSON, C.F. 1973. Juvenile hormone analogs: a possible case of mistaken identity? *Science* 181:672–674.

SLADE, M., and WILKINSON, C.F. 1974. Degradation and conjugation of Cecropia juvenile hormone by the southern armyworm (*Prodenia eridania*). *Comp. Biochem. Physiol.* 49B:99–103.

SLADE, M., and ZIBITT, C.H. 1971. Metabolism of Cecropia juvenile hormone in lepidopterans, pp. 45–48, *in* A.S. Tahori (ed.). Chemical Releasers in Insects, Proc. 2nd IUPAC Congr. Pestic. Chem., Vol. 3. Gordon and Breach, New York.

SLADE, M., BROOKS, G.T. HETNARSKI, H.K. and WILKINSON, C.F. 1975. Inhibition of the enzymatic hydration of the epoxide HEOM in insects. *Pestic. Biochem. Physiol.* 5:35–46.

SLADE, M., HETNARSKI, H.K., and WILKINSON, C.F. 1976. Epoxide hydrase activity and its relationship to development in the southern armyworm, *Prodenia eridania*. *J. Insect Physiol.* 22:619–622.

SODERLUND, D.M. MESSEGUER, A., and BOWERS, W.S. 1980. Precocene II metabolism in insects: Synthesis of potential metabolites and identification of initial in vitro biotransformation products. *J. Agric. Food Chem.* 28:724–731.

SPARKS, T.C., and HAMMOCK, B.D. 1983. Insect growth regulators: Resistance and the future, pp. 615–668, *in* G.P. Georghiou and T. Saito (eds.). Pest Resistance to Pesticides. Plenum Press, New York.

TOMLIN, A.D. 1968. *trans*-Aldrin glycol as a metabolite of dieldrin in larvae of the southern house mosquito. *J. Econ. Entomol.* 61:855–857.

TULLOCH, A.P. 1963. Enzymatic production of (+)-threo-9,10-dihydroxyoctadecanoic acid in the spores of plant rusts. *Can. J. Biochem. Physiol.* 41:1115–1121.

VAN BEEK, T.A., and DEGROOT, A. 1986. Terpenoid antifeedants, part I. An overview of terpenoid antifeedants of natural origin. *Recl. Trav. Chim. Pays-Bas* 105:513–527.

WHITE, R.A., FRANKLIN, R.T., and AGOSIN, M. 1979. Conversion of α-pinene to α-pinene oxide by rat liver and the bark beetle *Dendroctonus terebrans* microsomal fractions. *Pestic Biochem. Physiol.* 10:233–242.

WING, K.D., SPARKS, T.C., LOVELL, V.M., LEVINSON, S.O., and HAMMOCK, B.D. 1981. The distribution of juvenile hormone esterase and its interrelationship with other proteins influencing juvenile hormone metabolism in the cabbage looper, *Trichoplusia ni*. *Insect Biochem.* 11:473–485.

WISNIEWSKI, J.R., RUDNICKA, M., and KOCHMAN, M. 1986. Tissue specific juvenile hormone degradation in *Galleria mellonella*. *Insect Biochem.* 16:843–849.

WIXTROM, R.N., and HAMMOCK, B.D. 1985. Membrane-bound and soluble-fraction epoxide hydrolases: Methodological aspects, pp. 1–93, *in* D. Zakim and D.A. Vessey (eds.). Biochemical Pharmacology and Toxicology, Vol. 1. John Wiley, New York.

YAMASAKI, R.B., and KLOCKE, J.A. 1987. Structure-bioactivity relationships of azadirachtin, a potential insect control agent. *J. Agric. Food Chem.* 35:467–471.

YU, S.J. 1986. Consequences of induction of foreign compound-metabolizing enzymes in insects, pp. 153–174, *in* L.B. Brattsten and S. Ahmad (eds.). Molecular Aspects of Insect–Plant Associations. Plenum Press, New York.

YU, S.J., 1987. Biochemical defense capacity in the spined soldier bug (*Podisus maculiventris*) and its lepidopterous prey. *Pestic. Biochem. Physiol.* 28:216–223.

YU, S.J., and HSU, E.L. 1985. Induction of hydrolases by allelochemicals and host plants in fall armyworm (Lepidoptera: Noctuidae) larvae. *Environ. Entomol.* 14:512–515.

YU, S.J., and TERRIERE, L.C. 1978a. Metabolism of juvenile hormone I by microsomal oxidases, esterase, and epoxide hydrase of *Musca domestica* and some comparisons with *Phormia regina* and *Sarcophaga bullata*. *Pestic. Biochem. Physiol.* 9:237–246.

YU, S.J., and TERRIERE, L.C. 1978b. Juvenile hormone epoxide hydrase in house flies, flesh flies and blow flies. *Insect Biochem.* 8:349–352.

YU, S.J., ROBINSON, F.A., and NATION, J.L. 1984. Detoxication capacity in the honey bee, *Apis mellifera* L. *Pestic. Biochem. Physiol.* 22:360–368.