oxidation had occurred at the ortho–meta positions, 3-hydroxy-4,4′-dichlorobiphenyl should have been the major rather than the minor metabolite of this PCB. As it is, the rate of 4,4′-dichlorobiphenyl metabolism and the predominance of the 4-hydroxy metabolite imply that the formation of an arene oxide between a chlorinated and an unchlorinated carbon atom proceeds quite readily so long as other substitution of the molecule does not restrict the approach of the enzyme.

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Esterase and Oxidase Activity of House Fly Microsomes Against Juvenile Hormone Analogues Containing Branched Chain Ester Groups and Its Induction by Phenobarbital

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Branched-chain esters of 11-methoxy-3,7,11-trimethyl-2,4-dodecadienoic acid and 3,7,11-trimethyl-2,4-dodecadienoic acid were compared as substrates for microsomal esterases prepared from the insecticide-susceptible CSMA strain of house flies. In vitro hydrolysis of the seven esters was very slight compared to that measured previously with straight-chain esters of the same group of compounds. The analogues were readily metabolized by microsomal oxidases of the CSMA strain and by those of an insecticide-resistant strain (Rutgers). Phenobarbital in the diet of adult house flies caused increased oxidative metabolism of the analogues, up to 16-fold in the CSMA strain and up to fourfold in the Rutgers strain. In bioassays conducted during the pupal stage, when microsomal oxidase activity is low compared to esterase activity, there was considerable difference in response to the seven analogues. Methoprene, the isopropyl ester of the 11-methoxy acid, was highly effective at 0.1 μg/pupa while some other analogues were ineffective at 10 μg/pupa. Since there was only a twofold difference among the analogues, in the rate of hydrolysis by the esterases, it is concluded that other factors determine the biological activity of these compounds.

In house fly bioassays of the morphogenic activity of juvenile hormone analogues of the alkyl-3,7,11-trimethyl-2,4-dodecadienoates, Henrick et al. (1976) found that of the four most toxic analogues, three were branched-chain esters such as isopropyl (methoprene ZR-515) and *sec*-butyl (ZR-644). In our study of the metabolism of six analogues of the same general type by housefly esterases, we found that methoprene was most resistant to these enzymes (Yu and Terriere, 1975). This result correlated well with morphogenic activity in bioassays performed at the same time. Similar results were obtained with blow flies and flesh flies, only methoprene being highly active in bioassays and highly resistant to esterase action (Terriere and Yu, 1977). These observations suggest that house fly esterases may be unable to accomodate compounds in which the ester function is a branched-chain alkyl and that resistance to esterase attack in vivo may be an important requirement for biological activity.

Compounds of the type mentioned above, including methoprene, are also converted to various oxidation products in the house fly (Quistad et al., 1975) and are metabolized by the microsomal oxidases of the house fly (Yu and Terriere, 1975, 1977) and of the blow fly and flesh fly (Terriere and Yu, 1977). The suitability of the various analogues as substrates for these oxidases seems to vary considerably but this may be only apparent since, under the conditions of assay for the oxidases, the esterases are also present and active. Furthermore, the oxidases also attack the products of the esterase, the JHA acids (Yu and Terriere, 1977), thus complicating the interpretation of assay results. Another aspect of these oxidative reactions is that phenobarbital, a well-known inducer of microsomal oxidases in the house fly (Yu and Terriere, 1973), has a

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stimulating effect with some analogues but not with others (Yu and Terriere, 1975). A plausible explanation of this result has not been found.

Through the generosity of the Zoecon Corporation, Palo Alto, Calif., we were supplied with most of the branched-chain esters tested by Henrick et al. (1976) for a further investigation of the relationship between hydrolysis by house fly esterases and biological activity. During these experiments we also renewed our study of the phenobarbital induction of microsomal oxidase action on these compounds. Our results are summarized here.

MATERIALS AND METHODS

Insects. Two strains of the house fly (*Musca domestica* L.) were used in these experiments. The CSMA strain is susceptible to insecticides and has a low level of microsomal oxidase activity. The Rutgers strain is resistant to diazinon and has a high level of microsomal oxidase activity (Philpot and Hodgson, 1971, 1972).

Enzyme Assays. The methods used in preparing microsomes and the in vitro incubation conditions were as described previously (Yu and Terriere, 1975). Microsomal JHA-esterase activity was determined by conversion of the hydrolytic products, 11-methoxy-3,7,11-trimethyl-2,4-dodecadienoic acid and 3,7,11-trimethyl-2,4-dodecadienoic acid, to their methyl esters followed by gas chromatography (Terriere and Yu, 1977). Microsomal JHA-oxidase activity was determined by the substrate disappearance method using GC analysis (Terriere and Yu, 1973). All incubations were in triplicate.

Treatment of Flies. When sodium phenobarbital (USP grade) was used as an inducing agent, groups of 1-day old female adults were fed a diet of sucrose, powdered nonfat milk, and powdered egg yolk (12:12:1) containing the test compound for 3 days prior to preparation of microsomes (Yu and Terriere, 1973).

The bioassays were performed with newly molted house fly pupae. Pupae were topically treated on the abdomen with 1 μL of acetone solution of the JH analogues. Treated pupae were held in glass jars at 24 °C. The activity of the compounds was measured by the number of flies emerging successfully from the puparium.

JH Analogues. The following JH analogues were provided by the Zoecon Corporation, Palo Alto, Calif: ZR-485 (isopropyl 3,7,11-trimethyl-2,4-dodecadienoate), 77.7% 2*E*,4*E* isomer and 22.3% 2*Z*,4*E* isomer; ZR-515, methoprene, (isopropyl 11-methoxy-3,7,11-trimethyl-2,4-dodecadienoate), 95.2% 2*E*,4*E* isomer and 4.8% 2*Z*,4*E* isomer; ZR-629 (isobutyl 3,7,11-trimethyl-2,4-dodecadienoate), 78.4% 2*E*,4*E* isomer and 21.6% 2*Z*,4*E* isomer; ZR-633 (*sec*-butyl 3,7,11-trimethyl-2,4-dodecadienoate), 81.5% 2*E*,4*E* isomer and 18.5% 2*Z*,4*E* isomer; ZR-644 (*sec*-butyl 11-methoxy-3,7,11-trimethyl-2,4-dodecadienoate), 81.1% 2*E*,4*E* isomer and 18.9% 2*Z*,4*E* isomer; ZR-738 (isopentyl 11-methoxy-3,7,11-trimethyl-2,4-dodecadienoate), 83.2% 2*E*,4*E* isomer and 16.8% 2*Z*,4*E* isomer; ZR-753 (*sec*-butenyl 11-methoxy-3,7,11-trimethyl-2,4-dodecadienoate), 75.5% 2*E*,4*E* isomer and 24.5% 2*Z*,4*E* isomer. All structures are shown in Figure 1.

RESULTS AND DISCUSSION

House fly microsomes possess about twice as much JHA-esterase activity as other fractions of the cell (Yu and Terriere, 1975) and were used as enzyme source in this study. Microsomes from abdomens of female adults of the CSMA strain were incubated with the seven JH analogues shown in Figure 1. Hydrolysis was very slow, only 1.9–5.3 nmol of the JH analogues metabolized in 3 h by the microsomes from 25 abdomens (Table I). These activities

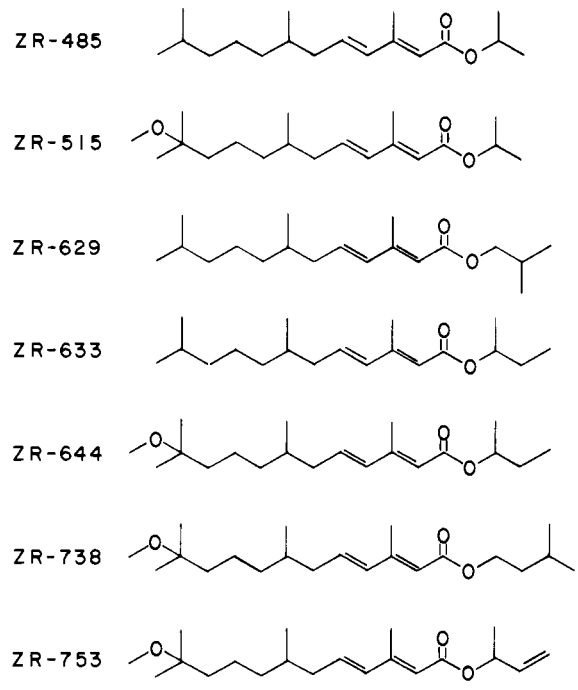

Figure 1. Structures of juvenile hormone analogues.

Table I. Metabolism of JH Analogues by Microsomal Esterases from CSMA House Fly Adults

JHA	JHA metabolized, nmol (25 abdomens)$^{-1}$ (3 h)$^{-1}$ [a]
ZR-485	1.9 ± 0.4
ZR-515	2.0 ± 0.5
ZR-629	5.3 ± 0.2
ZR-633	2.4 ± 0.3
ZR-644	2.7 ± 0.4
ZR-738	5.3 ± 0.7
ZR-753	4.3 ± 0.2

[a] Mean ± SD of three determinations. Calculation based on 2*E*,4*E* isomer only. Enzyme from 8-day old females.

are approximately $^1/_{40}$ those observed when straight-chain esters of the same group of analogues were incubated with microsomes from the closely related NAIDM strain (Yu and Terriere, 1975). Apparently the branched-chain esters are poor substrates for these enzymes. There is some indication that branching adjacent to the ester function presents a bigger obstacle to enzyme attack than when it occurs at a more distant position, e.g., compounds ZR-629 and ZR-738. This relationship does not hold, however, in the case of the *sec*-butenyl ester, ZR-753. In the earlier study we were unable to detect esterase activity against methoprene (ZR-515) whereas in these experiments some hydrolysis did occur. This difference is probably due to our use of a more direct method of assay, i.e., measurement of the acid produced rather than measurement of the substrate utilized.

These JH analogues are known to be oxidatively metabolized in the housefly, the attack occurring at one of the double bond positions and at the C-11 methoxy group, if present (Quistad et al., 1975; Yu and Terriere, 1977). Secondary metabolism of the products and of the corresponding acid produced by the esterases also occurs (Yu and Terriere, 1977). Thus, in order to gauge the role of these oxidases in the metabolism of such compounds, it is necessary to use the substrate disappearance method of assay. Furthermore, since the esterases are also active during the incubations, the contribution of the oxidases

Table II. Metabolism of JH Analogues by Microsomal Oxidases from Adult House Flies and Induction by Phenobarbital

JHA	JHA metabolized, nmol (25 abdomens)$^{-1}$ (3 h)$^{-1}$[a]			
	CSMA		Rutgers	
	Control	Phenobarbital	Control	Phenobarbital
ZR-485	6.1 ± 5.4	101.1 ± 20.3	37.5 ± 6.8	142.5 ± 9.9
ZR-515	24.2 ± 13.0	164.2 ± 8.4	79.9 ± 9.2	231.3 ± 7.3
ZR-629	11.2 ± 5.1	72.4 ± 7.8	50.4 ± 7.2	85.4 ± 7.6
ZR-633	9.2 ± 7.8	35.7 ± 6.8	53.5 ± 9.8	87.6 ± 11.8
ZR-644	10.4 ± 2.5	71.9 ± 10.3	33.2 ± 11.4	137.9 ± 4.3
ZR-738	10.0 ± 6.5	35.2 ± 10.3	19.5 ± 5.6	47.4 ± 7.5
ZR-753	20.3 ± 5.5	105.1 ± 5.2	64.7 ± 0.7	128.8 ± 4.7

[a] Mean ± SD of three determinations. One-day-old females were fed a diet containing 1% sodium phenobarbital for 3 days prior to enzyme assays. Calculation based on both isomers.

must be estimated by the difference between incubations with NADPH and those without this cofactor. Endogenous oxidative metabolism is negligible when NADPH is not added to the system.

The results of experiments with the microsomal oxidases are presented in Table II. It is seen that the oxidases of the CSMA strain are considerably more active in the metabolism of the JH analogues than the esterases, the difference ranging from twofold (ZR-629) to 12-fold (ZR-515). The structure of the ester group does not appear to be a factor in the activity of the enzyme.

The microsomal oxidases of the Rutgers strain were two–six times more active than those of the CSMA strain in the metabolism of the seven analogues. This was expected from earlier reports that insecticide-resistant house flies are more tolerant of certain JH analogues (Cerf and Georghiou, 1974; Vinson and Plapp, 1974). There is a rough parallel between the two strains in the greater activity of their enzymes toward ZR-515 and ZR-753, but this does not extend to the more slowly metabolized analogues, ZR-738 being most resistant to Rutgers strain microsomes whereas ZR-485 is most resistant to the CSMA enzymes.

Phenobarbital in the diet of adult house flies for 3 days had a powerful effect on the microsomal oxidase activity of both strains, increasing the metabolic rate as much as 16-fold in CSMA and fourfold in the Rutgers strain. This drug had no effect on the activity of the JHA-esterases, confirming our previous findings (Yu and Terriere, 1975; 1977).

In the earlier study of the induction of oxidative metabolism of such compounds by phenobarbital, we found increased metabolism of three analogues (ZR-512, ZR-515, ZR-619) but no increase in three others of similar structure (ZR-520, ZR-699, and ZR-777) (Yu and Terriere, 1975). The results of the present investigation offer an explanation for this apparent variation in the phenobarbital effect. In this case there was very little esterase activity and thus little competition with the oxidases for the JHA substrates. In the previous study there was substantial esterase activity, 132, 230, and 240 nmol of substrate metabolized (30 abdomens)$^{-1}$ (3 h)$^{-1}$ for ZR-520, ZR-699, and ZR-777, respectively, compared to oxidase activities of 59, 22, and 11 nmol with the same three substrates. It seemed likely, therefore, that any increased oxidase activity due to induction might have been obscured by the high esterase activity.

To test this hypothesis we performed the following experiment: microsomes from phenobarbital-induced adults (CSMA strain) were incubated with ZR-520 (ethyl 11-methoxy-3,7,11-trimethyl-2,4-dodecadienoate) in the presence of an NADPH-generating system and 10^{-4} M paraoxon. Under these conditions there was a 64% inhibition of the esterase and a 16-fold increase (average of two experiments) in the oxidative metabolism of the JH analogue. Although we did not perform similar tests with ZR-699 and ZR-777, the evidence with ZR-520 indicates that the metabolism of these analogues would also be increased by the phenobarbital exposure. These findings convince us that phenobarbital does induce the oxidative metabolism of all of the JH analogues studied so far.

Table III. Biological Activity of JH Analogues against CSMA House Fly Pupae

JHA	% pupal-adult ecdysis at indicated dose, μg/pupa[a]		
	0.1	1	10
ZR-485	94	29	0
ZR-515	0	0	0
ZR-629	100	100	100
ZR-633	100	69	54
ZR-644	38	33	14
ZR-738	100	97	31
ZR-753	100	100	100

[a] Groups of 20 newly molted pupae were treated topically with the compounds in 1 μL of acetone. Average of two experiments.

The morphogenic potency of the seven analogues was measured by the topical treatment of newly molted pupae of the CSMA strain as described in the Methods section. This method differs slightly from that of Henrick et al. (1975) who treated late third instar larvae instead of white pupae, but the results (Table III) are in general agreement with theirs with respect to relative activities. We differ only in the positions of ZR-738 and ZR-753 which are reversed from their rankings.

At 1 μg/pupa (Table III) there is a correlation between biological activity and resistance to esterase attack (Table I). This relationship also holds at a dose of 10 μg/pupa except in the case of ZR-738. The results are not very convincing, however, because of the generally low esterase activity against all substrates.

It is not likely that these differences in biological activity are due to differences in rates of microsomal oxidation because during the pupal stage oxidase activity is very low (Yu and Terriere, 1971, 1974). Also, as seen in Table II, there is not much difference in the rate at which the seven analogues are metabolized by these enzymes.

This enzyme system is quite active during the midpart of the third instar, however, and throughout the adult stage of the house fly (Yu and Terriere, 1971, 1974). It is likely, therefore, that oxidative degradation of such JH analogues encountered earlier in the larval stage or as adults would be a factor of some importance in their action. There is evidence of this in the higher activity of the microsomal oxidases of the Rutgers strain and the fact, mentioned earlier, that such strains exhibit resistance to these compounds.

The study of esterolytic and oxidative metabolism of the dodecadienoate juvenile hormone analogues by enzymes isolated from the house fly now includes 12 compounds. The results thus far permit certain conclusions to be drawn: all of the compounds are susceptible to metabolism by the microsomal oxidases with no clear evidence of structural preference or of influence on biological activity (except as noted above in connection with resistance); their resistance to hydrolysis by the microsomal esterases varies immensely and this can be correlated with biological activity in the case of the isopropyl esters, i.e., the compounds which resist hydrolysis are probably more potent as a result; this factor is not enough to provide potency with the other compounds studied, however, showing that other conditions must also be met. These conditions are probably related to cuticle penetration, transport within the insect, and conformation with the receptor site.

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Extensive Degradation of Silvex by Synergistic Action of Aquatic Microorganisms

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The herbicide 2-(2,4,5-trichlorophenoxy)propionic acid (silvex) was extensively degraded by a mixed culture of a *Pseudomonas* species and an *Achromobacter* species isolated from farm pond water. Neither of the two organisms separately was able to metabolize the herbicide. In the degradation of silvex by the mixed culture, chlorine was liberated from the herbicide, the aromatic ring was cleaved, and CO_2 was evolved. Except for a minor amount of 2,4,5-trichlorophenol, no silvex metabolites as detected by TLC were found. The mixed culture also readily decomposed 2,4,5-trichlorophenol and 3,5-dichlorocatechol.

The herbicide 2-(2,4,5-trichlorophenoxy)propionic acid (silvex) is used for controlling certain aquatic weeds (Blackburn, 1963; Frank et al., 1963). The metabolic fate of silvex in the aquatic environment is of obvious concern because of the potential toxicity of the herbicide and its metabolites to nontarget organisms and their possible adverse effects on man through his drinking-water supplies. Among the factors which determine the fate of a chemical in natural ecosystems, microbial transformation is one of the most important. Reports on microbial degradation of silvex are few. It has been reported that silvex and a structurally similar herbicide, 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), are very resistant to microbial degradation (Alexander and Aleem, 1961; Burger et al., 1962). The recalcitrant nature of these chemicals was attributed to the number of chlorines attached to the aromatic nucleus and to the presence of chlorine on the meta position of the ring. A soil fungus, *Streptomyces viridochromogenes*, was capable of cleaving the ether linkage and oxidizing the propionic acid moiety of silvex, but could not degrade the remaining 2,4,5-trichlorophenol (Bounds and Colmer, 1965). A *Brevibacterium* sp. was shown to cometabolize 2,4,5-T to 3,5-dichlorocatechol without any further alteration (Horvath, 1971). In this paper, we report that silvex, a molecule generally considered as recalcitrant, is extensively degraded by the synergistic action of two species of aquatic microorganisms.

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MATERIALS AND METHODS

Isolation of Aquatic Microorganisms. A population of aquatic microorganisms capable of degrading silvex was developed by an enrichment culture technique using farm pond water as an initial source of inoculum. The enrichment medium contained K_2HPO_4, 4.8 g; KH_2PO_4, 1.2 g; NH_4NO_3, 0.5 g; $MgSO_4 \cdot 7H_2O$, 0.2 g; $Ca(NO_3)_2 \cdot 4H_2O$, 0.04 g; $Fe_2(SO_4)_3$, 0.001 g; yeast extract (Difco), 2.0 g; silvex, 0.3 g per liter of distilled water. The pH of the medium was 7.3. Silvex degradation was determined by measuring chloride ion release in the medium (Iwasaki et al., 1952) and by assessing the loss of UV absorbance of the supernatant fluid at 288 nm (λ max for silvex). Once significant loss of silvex became evident, an aliquot from the inoculated flask was transferred to a flask containing the fresh enrichment medium and the procedure was repeated several times. After several transfers, the enriched culture solution was streaked on plates consisting of basal mineral medium and agar supplemented with 300 ppm of silvex and 0.2% yeast extract. The effective organisms were maintained on the basal mineral medium supplemented with 300 ppm of silvex and 0.2% yeast extract.

Degradation of Silvex by Aquatic Microorganisms. To examine the time-course of silvex degradation, the