

Inhibition of Cytochrome P450 6D1 by Alkynylarenes, Methylenedioxyarenes, and Other Substituted Aromatics

Jeffrey G. Scott,* Maryam Foroozesh,† Nancy E. Hopkins,‡ Timothy G. Alefantis,* and William L. Alworth§

*Department of Entomology, Comstock Hall, Cornell University, Ithaca, New York 14853; †Department of Chemistry, Xavier University, New Orleans, Louisiana 70125; ‡Department of Chemistry, Millsaps College, Jackson, Mississippi 39210; and §Department of Chemistry, Tulane University, New Orleans, Louisiana 70118

Received September 24, 1999, accepted January 6, 2000

We evaluated 33 compounds, comprising five different structural groups (alkynylpyrenes, alkynylphenanthrenes, methylenedioxyarenes, flavones, and miscellaneous), as inhibitors of house fly P450 6D1 (CYP6D1). In general, alkynylpyrenes were the most potent group of inhibitors, with maximum effectiveness noted when the substituent was in the 4 position. Substituted phenanthrenes were reasonable CYP6D1 inhibitors with the lowest IC_{50} observed for the analogue with the methylenedioxy substituent between the 9 and 10 positions. The 10 methylenedioxyarenes varied considerably in their ability to inhibit CYP6D1. Piperonyl butoxide was the most potent inhibitor with increasing length of the alkyl substituent resulting in decreasing inhibition. Karanjin had the lowest IC_{50} of the 4 flavones that were tested. Overall, the most potent CYP6D1 inhibitors were large planar compounds. Four inhibitors were evaluated as permethrin synergists in the LPR strain of house fly (permethrin is detoxified via CYP6D1 in this strain) and they increased permethrin toxicity by 16- to 83-fold. The effect of structure on inhibition potency, as well as the mechanism of inhibition for seven representative inhibitors, is discussed. © 2000 Academic Press

INTRODUCTION

The cytochrome P450 monooxygenases are important in the metabolism of many endogenous and exogenous compounds. The P450 monooxygenases of insects have several functional roles, including growth, development, resistance to pesticides, and tolerance to plant toxins (1–3). Reasons for the large number of substrates that can be metabolized by this enzymatic system are the multiple isoforms of P450s that exist in each organism and the broad substrate specificity of some isoforms (4).

Isoform-specific inhibitors identified for mammalian P450s have been useful for *in vivo* and *in vitro* studies and hold potential for therapeutic modulation of human drug metabolism (5). Identification of isoform-specific inhibitors of P450s in insects could prove useful as *in vivo* and *in vitro* probes and could lead to the identification of selective insecticide synergists. Although many insecticide synergists have been

identified and the mechanisms of inhibition elucidated for certain classes of synergists (6–9), evaluation of the inhibitors of an individual insect P450s has received little attention.

Alkynylarenes and methylenedioxyarenes are well-documented inhibitors of P450s (7, 10). The alkynylarenes have been well studied as inhibitors of mammalian P450s, although they have not been examined as inhibitors of individual insect P450s. Methylenedioxyarenes are well-known inhibitors of insect P450s, some of these compounds proving to be useful insecticide synergists (7, 9). Although there has been substantial work on the structure–activity of methylenedioxyarenes as insecticide synergists (11, 12), no such study has been undertaken with a P450 isoform-specific assay.

CYP6D1 is a P450 isoform that is responsible for monooxygenase-mediated pyrethroid resistance in the Learn-PyR (LPR) strain of house fly (13–15). CYP6D1 (P450_{LPR}) has been purified (16) and a monospecific antisera developed (17).

Using this antisera it was shown that methoxyresorufin *O*-demethylation (MROD) is a CYP6D1-specific assay in LPR microsomes (18). *CYP6D1* has been sequenced (19) and cloned (20) and potential substrates (21), inducers (22), and a group of miscellaneous inhibitors (23) have been evaluated. In this study, 33 compounds comprising five different structural groups (alkynylpyrenes, alkynylphenanthrenes, methylenedioxyarenes, flavones, and miscellaneous) were evaluated as inhibitors of a CYP6D1-specific monooxygenase activity in house fly microsomes.

MATERIALS AND METHODS

House Flies and Preparation of Microsomes

Learn-PyR is a multiresistant strain of house fly that is highly resistant to pyrethroid insecticides (24). Cornell-S is an insecticide-susceptible strain (22). Flies were reared by standard methods (16) on larval medium consisting of 510 g calf-manna pellets (Manna Pro Corporation, St. Louis, MO), 25 g dried active Bakers yeast (ICN Pharmaceuticals Inc., Costa Mesa, CA), 110 g wood chips (Northeastern Products, Warrensburg, NY), 790 g wheat bran (Agway, Syracuse, NY), and 1900 ml of water.

Microsomes were prepared from abdomens of 200 female LPR house flies as described previously (25). This method involves homogenizing isolated abdomens in 0.1 M sodium phosphate buffer (pH 7.5) containing 10% glycerol, 1 mM EDTA, 0.1 mM dithiothreitol, 1 mM 1-phenyl-2-thiourea, and 0.1 mM phenylmethylsulfonyl fluoride and centrifugation at 10,000g for 20 min. The resulting supernatant was centrifuged at 100,000g for 60 min. This pellet was resuspended in buffer (25) and used as the enzyme source. Protein was assayed (26) using bovine serum albumin as the standard.

Chemicals

The syntheses of 1-ethynylpyrene (1EP), 2-ethynylpyrene (2EP), 2-ethynylanthracene (2EA), (27), 2-ethynylphenanthrene (2EPH), 3-ethynylphenanthrene (3EPH), 1-(1-propynyl)pyrene (1PP),

4-(1-propynyl)biphenyl (4PBi), 9-ethynylphenanthrene (9EPH), 2-(1-propynyl)phenanthrene (2PPH), 4-ethynylpyrene (4EP) (28), and 1-adamantanol (1APE) (29) were described previously. Sphondin was provided by Dr. M. Berenbaum (Univ. of Illinois), karanjin and flavanone were from Indofine Chemical (Belle Mead, NJ), α -naphthoflavone (α -NF) and flavone were from Sigma (St. Louis, MO), malathion was from Chem Service (West Chester, PA), parathion was from American Cyanamid (Princeton, NJ), 9,10-methylenedioxyphenanthrene (PD) was from Fundamental Research (Berkeley, CA), verbutin (MB-599,(30)) was from Dr. L. Pap (CHINNOIN AgChem, Hungary), and methylenedioxyphenyl compounds having different length alkyl chains were obtained from Dr. C. Marcus (C₀MDP, C₃MDP, and C₈MDP; Univ. of New Mexico) or Dr. C. Wilkinson (C₁₂MDP and C₆MDP). All compounds were dissolved in acetone or dimethyl sulfoxide.

Synthesis of New Compounds

1-Ethynyladamantane (1EAd) was prepared from 1-adamantyl methyl ketone (Aldrich Chemical Co., Milwaukee, WI) by the method described previously (28). The crude product was purified by flash column chromatography on silica gel with petroleum ether as solvent. GC/MS showed that the white, waxy solid obtained, mp 91–93°C, was 99% pure. ¹H NMR (CDCl₃): δ 1.67–1.68 (s,6H), 1.88–1.95 (d,6H), 1.95 (s,3H), 2.1 (s,1H,ethynyl); ¹³C NMR (CDCl₃): δ 28.00 (3C), 29.80 (1C), 36.12 (3C), 42.40 (3C), 66.40 (1C), 93.20 (1C). Spectra were obtained with a GE Omega 400-mHz multinuclear NMR spectrometer.

1,2,3,4,6,7,8-Hexahydroxyrene (8.09 g, 0.039 mol, Aldrich) dissolved in 100 ml of dry tetrachloroethylene was cooled to –20°C under N₂ and 4 eq of AlCl₃ and 1.2 eq of acetyl chloride were added. The reaction mixture was stirred for 3 h at –20°C under N₂ and allowed to warm slowly to room temperature, and the reaction quenched by the addition of 100 ml of ice-cold 20% HCl (v:v). The organic layer was separated

and the aqueous acid extracted twice with an equal volume of methylene chloride. The organic extracts were combined, washed with dilute KOH solution and twice with water, and then dried over solid MgSO_4 . The organic solvent was then evaporated under a vacuum and the 4-acetyl-1,2,3,6,7,8-hexahydropyrene product aromatized to 4-acetylpyrene and then converted to 4-propynylpyrene by procedures previously described (28). The yield of pale-yellow crystalline 4PP, mp 103–106°C, after purification by flash chromatography on silica gel with petroleum ether as solvent, was 35%. GC/MS showed that the 4PP was >99% pure. ^1H NMR (CDCl_3): δ 2.31 (s, 3H, methyl), 7.97–8.26 (m, 7H, ArH), 8.26 (s, 1H), 8.67–8.69 (d, 1H). ^{13}C NMR (CDCl_3): δ 47.8 (1C, CH_3), 96.8 (1C), 96.9 (1C), 121.1–131.5 (16C, ArC).

Inhibition of CYP6D1

Methoxyresorufin *O*-demethylation (MROD) assays, which are specific for CYP6D1 in LPR microsomes (18), were run using an Aminco SPF-500 spectrofluorometer at 32°C as described previously (31) with the following modifications (23). Solution (1 ml) containing inhibitor (in 1 μl of acetone), NADPH (0.1 mM), 0.1 mg of microsomal protein, and buffer was incubated at room temperature for 60 s. Preliminary studies indicated this was sufficient time for maximum inhibition of MROD activities (i.e., inhibition was not enhanced by increasing incubation times from 1 min up to 5 min). A solution (1 ml) containing buffer, NADPH (0.1 mM), and fluorimetric substrate (4 μl of 1.0 mM methoxyresorufin (Molecular Probes, Eugene, OR) in DMSO was then added to the cuvette and thoroughly mixed. Reactions were unaffected by 1 μl of acetone (control) and were linear for at least 5 min under these conditions. Samples without NADPH showed no activity. A series of concentrations (5–8) was tested for each compound with a given batch of microsomes. Results for each compound are the average of at least three separate experiments using different batches of microsomes. The effect of preincubation with NADPH on inhibition was determined

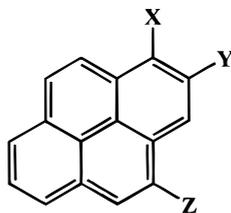
for representative compounds using a CytoFluor 4000 following a 10-min incubation at 25°C. Results for each compound are the average of at least three separate experiments using different batches of microsomes.

Synergism

Permethrin or putative synergist was delivered in 0.5 μl acetone to the thoracic notum of female house flies (32). Synergists were delivered 1 h before the insecticide application at approximately their maximum sublethal dose (10 $\mu\text{g}/\text{fly}$ for karanjin, PD, and IEP; 2 $\mu\text{g}/\text{fly}$ for verbutin). Twenty 3- to 5-day-old house flies were treated for each dose. A minimum of three doses giving >0 and <100% mortality were used for each experiment. Each experiment was replicated at least three times. The treated insects were put in 200-ml Sweetheart ice cream cups covered with cheese cloth and held at 25°C. Each cup contained a piece of 4-cm dental wick soaked in 15% sugar water and the dental wicks were kept wet during the experiment. Mortality was assessed 24 h after insecticide application. Insects were considered dead if they were on their backs and unable to right themselves when disturbed. Bioassay data were pooled and analyzed based on standard probit analysis (33) as adapted to personal computer use (34), using Abbott's correction (35) for control mortality.

RESULTS AND DISCUSSION

Both ethynyl and propynyl substituted pyrenes were potent inhibitors of CYP6D1 (Fig. 1). The potency of inhibition was dependent upon the position of the substituent, with the greatest inhibition seen for the 4 position and the least for the 2 position. These compounds are among the most potent inhibitors of CYP6D1 identified to date, having IC_{50} 's comparable to xanthotoxin and chlorpyrifos (23). Given the inhibitory potency of the substituted pyrenes and the high turnover number for CYP6D1-mediated benzo[*a*]pyrene metabolism (14), it is clear that the active site of CYP6D1 will readily accommodate large planar aromatic compounds. The weak inhibitory effect of ethynyl substituted



| X | Y | Z | compound | I_{50} [M] ^a |
|--------------------|------|--------------------|----------|----------------------------|
| H | H | -CCH | 4EP | $1.8 (0.6) \times 10^{-8}$ |
| H | H | -CCCH ₃ | 4PP | $3.8 (1.3) \times 10^{-8}$ |
| -CCCH ₃ | H | H | 1PP | $7.3 (3.3) \times 10^{-8}$ |
| -CCH | H | H | 1EP | $1.5 (0.1) \times 10^{-7}$ |
| H | -CCH | H | 2EP | $3.7 (1.0) \times 10^{-7}$ |

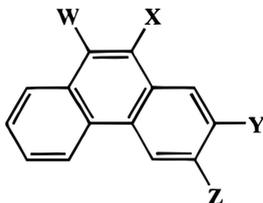
^a Values represent the mean (SE) for a minimum of three replications.

FIG. 1. Ethynyl and propynyl substituted pyrenes as inhibitors of cytochrome CYP6D1.

adamantane compounds (see below) emphasizes, however, that the active site readily accommodates only large ligands that are planar.

The substituted phenanthrenes varied in potency by nearly 100-fold (Fig. 2). The most effective inhibitor was PD, followed by 2PPh and 2EPh. There was no difference in I_{50} values for the latter two compounds, suggesting that both ethynyl and propynyl substituents were similar in activity. The 2-ethynyl substitution for phenanthrene gave the greatest inhibition of MROD activity, while substitution at the 3 or 9 positions was slightly less effective (Fig. 2).

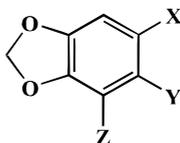
Methylenedioxyphenyl compounds have been extensively evaluated as insecticide synergists in house flies and other species (11, 12, 36), although the potency of these compounds against individual insect P450s has received little attention. Piperonyl butoxide and myristicin were the most potent of the methylenedioxyphenyl CYP6D1 inhibitors (Fig. 3). The length of the alkyl substitution caused considerable change in the inhibitory potency with $C_3H_7 > C_6H_{13} > H > C_8H_{17} > C_{12}H_{25}$ (Fig. 3). These results are similar to the potency of these compounds as insecticide synergists (12).



| W | X | Y | Z | compound | I_{50} [M] ^a |
|----------------------|------|--------------------|------|----------|----------------------------|
| -OCH ₂ O- | | H | H | PD | $4.3 (0.8) \times 10^{-8}$ |
| H | H | -CCCH ₃ | H | 2PPh | $8.7 (1.7) \times 10^{-7}$ |
| H | H | -CCH | H | 2EPh | $9.4 (3.2) \times 10^{-7}$ |
| H | H | H | -CCH | 3EPh | $2.4 (0.3) \times 10^{-6}$ |
| H | -CCH | H | H | 9EPh | $3.1 (1.0) \times 10^{-6}$ |

^a Values represent the mean (SE) for a minimum of three replications.

FIG. 2. Substituted phenanthrenes as inhibitors of cytochrome CYP6D1.



| X | Y | Z | compound | I_{50} [M] ^a |
|--|---|-------------------|---------------------------------|------------------------------|
| -CH ₂ CH ₂ CH ₃ | -(CH ₂ OCH ₂) ₃ CH ₂ CH ₂ CH ₃ | H | piperonyl butoxide ^b | 4.3 (2.1) x 10 ⁻⁷ |
| -CH ₂ CHCH ₂ | H | -OCH ₃ | myristicin | 6.5 (0.8) x 10 ⁻⁷ |
| -CH ₂ CH ₂ CH ₃ | H | H | C ₃ MDP | 1.4 (0.5) x 10 ⁻⁶ |
| -CHCHCH ₂ CH ₃ | H | H | isosafrrole ^b | 3.4 (1.0) x 10 ⁻⁶ |
| <i>n</i> -hexyl | H | H | C ₆ MDP | 7.3 (3.7) x 10 ⁻⁶ |
| -C(CH ₃) ₂ OCH ₂ CCCH ₃ | H | H | verbutin | 1.6 (0.6) x 10 ⁻⁵ |
| H | H | H | C ₀ MDP | 1.7 (0.5) x 10 ⁻⁵ |
| H | -(CH ₂ OCH ₂) ₃ CH ₂ CH ₂ CH ₃ | H | M1 ^b | 6.6 (1.6) x 10 ⁻⁵ |
| <i>n</i> -octyl | H | H | C ₈ MDP | ~10 ⁻⁵ |
| <i>n</i> -dodecyl | H | H | C ₁₂ MDP | >10 ⁻⁵ |

^a Values represent the mean (SE) for a minimum of three replications.

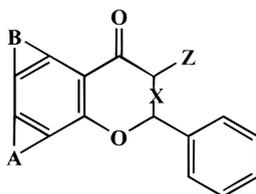
^bData from Scott 1996.

FIG. 3. Substituted methylenedioxyphenyls as inhibitors of cytochrome CYP6D1.

Flavone was approximately 10-fold more potent an inhibitor than flavanone, indicating that the double bond in the central ring structure of these analogues is important for inhibition (Fig. 4). This observation also emphasizes the apparent preference of CYP6D1 to bind planar inhibitors. The similar I_{50} values for β -NF and α -NF, in comparison to flavone, indicate that these substitutions at the 5,6 or 7,8 positions

have little effect on inhibitory potency (Fig. 4). The most potent CYP6D1 inhibitor within this series was karanjin with an I_{50} of 6.0×10^{-8} M (Fig. 4).

Two organophosphates, malathion and para-thion, were tested (Fig. 5). Both were found to be substantially less potent CYP6D1 inhibitors than a previously tested organophosphate (chlorpyrifos $I_{50} = 3.7 \times 10^{-8}$ M, (23)). Previously,



| A | B | X | Z | compound | I_{50} [M] ^a |
|---------------|---------------|-------|------------------|--------------------------------------|------------------------------|
| 2H | 2H | C=C | OCH ₃ | karinjine | 6.0 (0.5) x 10 ⁻⁸ |
| -CH=CH-CH=CH- | 2H | C=C | H | α -naphthoflavone | 2.8 (0.8) x 10 ⁻⁷ |
| 2H | 2H | C=C | H | flavone | 3.1 (0.5) x 10 ⁻⁷ |
| 2H | -CH=CH-CH=CH- | C=C | H | β -naphthoflavone ^b | 3.3 (0.1) x 10 ⁻⁷ |
| 2H | 2H | CH-CH | H | flavanone | 2.6 (1.1) x 10 ⁻⁶ |

^a Values represent the mean (SE) for a minimum of three replications.

^bData from Scott 1996.

FIG. 4. Flavanone and substituted flavones as inhibitors of cytochrome CYP6D1.

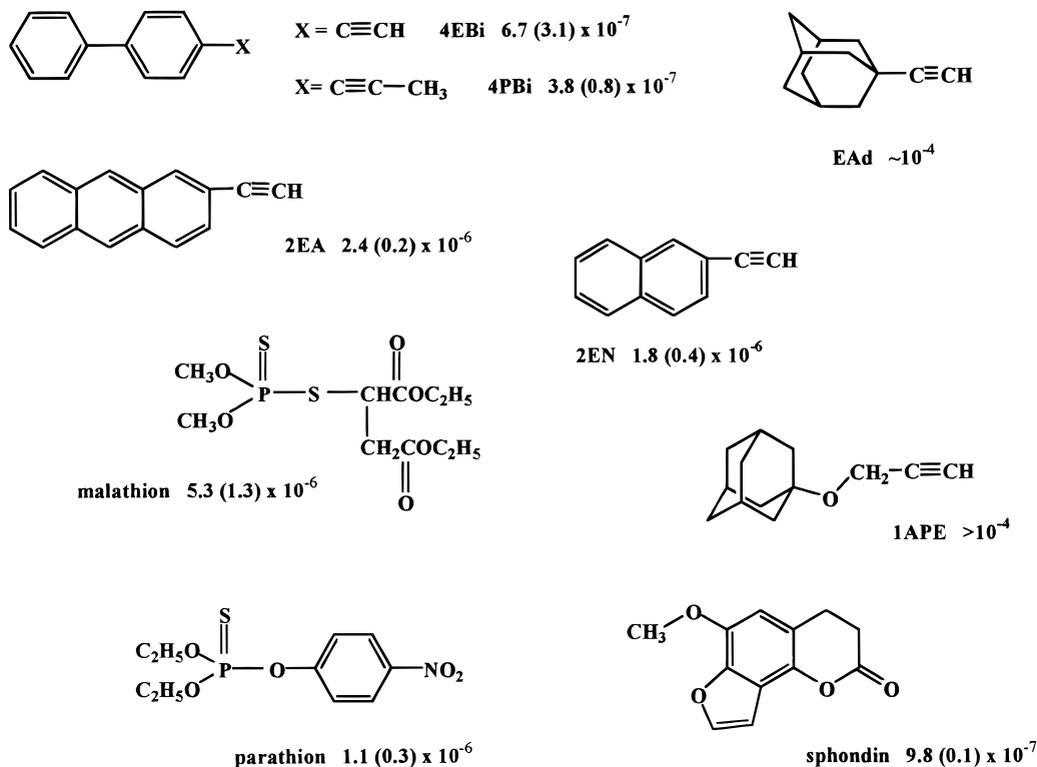


FIG. 5. Miscellaneous compounds as inhibitors of cytochrome CYP6D1. IC_{50} values (molar) represent the mean (SE) for a minimum of three replications.

it has been shown that the ability of organophosphates to inhibit acetylcholinesterase (the target site responsible for their insecticidal activity) is due, at least in part, to the electronegativity of the central phosphate atom. Whether the same properties or other factors (such as hydrophobicity) make organophosphates better inhibitors of CYP6D1 is an interesting question for further study.

Similar I_{50} values for 4-EBi and 4-PBi indicate that both ethynyl and propynyl substituents of biphenyl have similar inhibitory potency toward CYP6D1 (Fig. 5). The substituted biphenyls were slightly better inhibitors than ethynyl substituted anthracene or naphthalene (Fig. 5). EAd and IAPE were poor inhibitors (Fig. 5). This is consistent with our other results indicating that planar molecules were generally better inhibitors of CYP6D1.

Furanocoumarins are plant allelochemicals

found in at least eight plant families. They are highly toxic to a wide variety of organisms, including bacteria, plants, insects, fish, birds, and mammals, because they are capable of reacting directly and irreversibly with pyrimidine bases in DNA after photoactivation (37, 38). Two types of furanocoumarins, linear (e.g., xanthotoxin and bergapten) and angular (e.g., angelicin and spondin), occur in plants. In the case of CYP6D1, the linear furanocoumarins xanthotoxin and 5-methoxypsoralen were 2- to 61-fold more potent inhibitors (23) compared to the angular furanocoumarin spondin (Fig. 5).

One practical application for identifying CYP6D1 inhibitors is for the identification of novel insecticide synergists. For example, piperonyl butoxide and isosafrole are inhibitors of CYP6D1 and they enhance the toxicity of the pyrethroid insecticide permethrin by 800-fold (39) and 130-fold (23), respectively, in the LPR

TABLE 1
Toxicity of Permethrin \pm CYP6D1 Inhibitors to LPR
House Flies by Topical Application

| Inhibitor | Permethrin LD ₅₀ ^a | SR ^b |
|-----------|--|-----------------|
| None | 50,000 | — |
| Verbutin | 601 | 83 |
| PD | 1320 | 38 |
| IEP | 2660 | 19 |
| Karanjin | 3040 | 16 |

^a LD₅₀ in units of nanograms per fly.

^b Synergism ratio = LD₅₀ of permethrin/LD₅₀ of permethrin + inhibitor.

strain. Thus, four compounds that were identified in this paper as CYP6D1 inhibitors were evaluated as insecticide synergists. The LD₅₀ values of permethrin \pm CYP6D1 inhibitors are shown in Table 1. Verbutin was the best synergist within these compounds, giving an 83-fold increase in permethrin toxicity. PD, IEP, and karanjin were also found to be synergists, causing a 16- to 38-fold increase in permethrin toxicity to LPR house flies (Table 1).

A general method for testing whether P450 inactivation is mechanism-based (i.e., suicide inhibition) is to examine the effect of preincubation of the inhibitor with NADPH (and O₂) prior to the addition of substrate (28, 40). Inhibition of CYP6D1 activity was enhanced by preincubation with NADPH for piperonyl butoxide and parathion, but not for karanjin, IEP, xanthotoxin, chlorpyrifos, or chlorpyrifos oxon (Table 2).

Piperonyl butoxide is well known as a P450 suicide substrate in microsomes (10) and our results are consistent with this idea. It is curious that parathion appears to be a mechanism-based inhibitor of CYP6D1 while another organophosphate insecticide (chlorpyrifos) was not. One possible explanation for this is that the putative intermediate formed during P450-mediated metabolism of organophosphates (41) could preferentially lead to inhibition (i.e., generation of the oxon and inactivation of the P450), as seen with parathion, or no inhibition (i.e., generation of thionophosphoric acid without inactivation of the P450), as was observed for chlorpyrifos.

The alkynylarenes tested and ranked as inhibitors of house fly CYP6D1 in this study have been previously investigated as selective inhibitors of P450 1A1, 1A2, and 2B1/2B2 in rat liver microsomes (27, 28), of P450 2b-10 in mouse lung and liver microsomes (42), of mammalian P450s 2B1, 2B4, 2B6, and 2B11 (43), and, most recently, of human P450 1A1, 1A2, and 1B1 expressed in *Escherichia coli* (40). These studies clearly established that the size and shape of the aromatic moiety of the alkynylarene, the position of substitution by the alkynyl group, and the presence of a terminal acetylene (ethyne) or a methyl acetylene (propyne) all affected the selectivity of the P450 inhibition observed.

As shown in Fig. 2, 9EPH was among the less effective alkynylarene inhibitors of CYP6D1.

TABLE 2
Effect of Preincubation with or without NADPH on Inhibition of CYP6D1-Mediated MROD Activity

| Inhibitor | Concentration (μ M) | Percentage of inhibition ^a | |
|--------------------|-----------------------------|---------------------------------------|--------------------------------|
| | | Preincubation with NADPH | Preincubation without NADPH |
| Parathion | 2.0 | 86 \pm 11 | 37 \pm 18 |
| Piperonyl butoxide | 1.0 | 95 \pm 13 | 58 \pm 9 |
| Xanthotoxin | 0.02 | 64 \pm 21 | 60 \pm 12 |
| Karanjin | 0.1 | 50 \pm 21 | 64 \pm 27 |
| Chlorpyrifos | 0.1 | 56 \pm 34 | 49 \pm 20 |
| Chlorpyrifos oxon | 50 | 90 \pm 9 | 86 \pm 17 |
| IEP | 0.5 | 73 \pm 35 | 82 \pm 40 |

^a Values represent the mean \pm SD.

Since 9EPh is consistently found to be the most potent inhibitor of members of the P450 2B subfamily (27, 28, 42, 43), the comparative data indicate that CYP6D1 differs significantly in active site geometry from members of the mammalian P450 2B subfamily. Conversely, the comparative inhibition data establish that house fly CYP6D1 shares some critical active site features with members of the mammalian P450 1A subfamily (including human P4501A1, and 1A2) and 1B1. For example, 4EP, 4PP, 1PP, and 1EP were found to be potent alkynylarene inhibitors of CYP6D1 (Fig. 1), and these same compounds are among the more effective inhibitors of P450 1A1, 1A2, and 1B1 (28, 40). However, propynes are more effective inhibitors of P450 1A1 than the corresponding ethynes (28, 40), while Figs. 1 and 2 illustrate that CYP6D1 is inhibited similarly by propynes and ethynes (4PP, 4EP; 1PP, 1EP; 2EPh, 2PPh). The strong inhibition of CYP6D1 by 4EP (Fig. 1) and the fact that 2EPh and 2PPh are nearly equally effective inhibitors of CYP6D1 (Fig. 2) indicate that the effects of alkynylarene inhibitors on CYP6D1 correspond more closely to the effects of these same alkynylarene inhibitors on human P450 1A2 than on human P450 1B1 (40).

Currently used insecticide synergists are general P450 inhibitors, effecting insect, vertebrate, and plant isoforms. Results from this study indicate that it should be possible to develop new insecticide synergists which inhibit insect P450s, but not those of mammals. Such inhibitors could be useful tools for enhancing the toxicity of insecticides to insects without increasing the toxicity to mammals.

ACKNOWLEDGMENTS

We thank C. Marcus for samples of C₀MDP, C₃MDP, and C₈MDP, M. Berenbaum for the sphondin, L. Pap for the verbutin, and F. Harrison for technical assistance. 1-Ethynyladamantane was originally prepared and studied by David H. Lee as part of his honors thesis in biological chemistry at Tulane University, May 1990. This research was supported in part by NIH Grants CA38192 (to WLA) and GM47835 (to JGS).

REFERENCES

1. E. Hodgson, Microsomal mono-oxygenases, in "Comprehensive Insect Physiology Biochemistry and Pharmacology" (G. A. Kerkut and L. C. Gilbert, Eds), vol. 11, p. 647, Pergamon Press, Oxford, 1985.
2. R. Feyereisen, Insect P450 enzymes, *Ann. Rev. Entomol.* **44**, 507 (1999).
3. J. G. Scott, N. Liu, and Z. Wen, Insect cytochromes P450: Diversity, insecticide resistance and tolerance to plant toxins, *Comp. Biochem. Physiol. C Pharmacol. Toxicol. Endocrinol.* (1998).
4. S. Rendic and F. J. Di Carlo, Human cytochrome P450 enzymes: A status report summarizing their reactions, substrates, inducers, and inhibitors, *Drug Metab. Rev.* **29**, 413 (1997).
5. J. R. Halpert, F. P. Guengerich, J. R. Bend, and M. A. Correia, Selective inhibitors of cytochromes P450, *Toxicol. Appl. Pharmacol.* **125**, 163 (1993).
6. R. L. Metcalf, T. R. Fukuto, C. Wilkinson, M. H. Fahmy, S. A. El-Aziz, and E. R. Metcalf, Mode of action of carbamate synergists, *J. Agric. Food Chem.* **14**, 555 (1966).
7. J. E. Casida, Mixed-function oxidase involvement in the biochemistry of insecticide synergists, *Agric. Food Chem.* **18**, 753 (1970).
8. C. F. Wilkinson, Insecticide synergists and their mode of action, in "Insecticide Resistance, Synergism, Enzyme Induction, Pesticide Chemistry," p. 117, Gordon & Breach, New York, 1971.
9. K. F. Raffa and T. M. Priestler, Synergists as research tools and control agents in agriculture, *J. Agric. Ent.* **2**, 27 (1985).
10. P. R. Ortiz de Montellano and M. A. Correia, Inhibition of cytochrome P450 enzymes, in "Cytochrome P450 Structure, Mechanism, and Biochemistry" (P. R. Ortiz de Montellano, Ed.), p. 305, Plenum Press, San Francisco, 1995.
11. P. S. Hewlett and C. F. Wilkinson, Quantitative aspects of the synergism between carbaryl and some 1,3-benzodioxole (methylenedioxyphenyl) compounds in houseflies, *J. Sci. Food Agric.* **18**, 279 (1967).
12. C. Wilkinson, R. L. Metcalf, and T. R. Fukuto, Some structural requirements of methylenedioxyphenyl derivatives as synergists of carbamate insecticides, *Agric. Food Chem.* **14**, 73 (1966).
13. G. D. Wheelock and J. G. Scott, The role of cytochrome P450_{pr} in deltamethrin metabolism by pyrethroid resistant and susceptible strains of house flies, *Pestic. Biochem. Physiol.* **43**, 67 (1992).
14. N. Liu and J. G. Scott, Genetic analysis of factors controlling elevated cytochrome P450, CYP6D1, cytochrome b₅, P450 reductase and monooxygenase activities in LPR house flies, *Musca domestica*, *Biochem. Genet.* **34**, 133 (1996).
15. P. J. Korytko and J. G. Scott, CYP6D1 protects thoracic ganglia of house flies from the neurotoxic insecticide

- cypermethrin, *Arch. Insect Biochem. Physiol.* **37**, 57 (1998).
16. G. D. Wheelock and J. G. Scott, Simultaneous purification of a cytochrome P-450 and cytochrome b₅ from the house fly *Musca domestica* L., *Insect Biochem.* **19**, 481 (1989).
 17. G. D. Wheelock and J. G. Scott, Immunological detection of cytochrome P450 from insecticide resistant and susceptible house flies (*Musca domestica*), *Pestic. Biochem. Physiol.* **38**, 130 (1990).
 18. G. D. Wheelock and J. G. Scott, Anti-P450_{IPR} antiserum inhibits specific monooxygenase activities in LPR house fly microsomes, *J. Exp. Zool.* **264**, 153 (1992).
 19. T. Tomita and J. G. Scott, cDNA and deduced protein sequence of *CYP6D1*: The putative gene for a cytochrome P450 responsible for pyrethroid resistance in house fly, *Insect Biochem. Mol. Biol.* **25**, 275 (1995).
 20. F. F. Smith and J. G. Scott, Functional expression of house fly (*Musca domestica*) cytochrome P450 *CYP6D1* in yeast (*Saccharomyces cerevisiae*), *Insect Biochem. Mol. Biol.* **27**, 999 (1997).
 21. J. G. Scott and G. D. Wheelock, Characterization of a cytochrome P450 responsible for pyrethroid resistance in the housefly, in "Molecular Mechanisms of Insecticide Resistance: Diversity Among Insects" (C. A. Mullin and J. G. Scott, Eds.), ACS Symposium Series 505, p. 16, ACS, Washington, DC, 1992.
 22. J. G. Scott, P. Sridhar, and N. Liu, Adult specific expression and induction of cytochrome P450_{IPR} in house flies, *Arch. Insect Biochem. Physiol.* **31**, 313 (1996).
 23. J. G. Scott, Inhibitors of CYP6D1 in house fly microsomes, *Insect Biochem. Mol. Biol.* **26**, 645 (1996).
 24. J. G. Scott and G. P. Georgioui, Rapid development of high-level permethrin resistance in a field-collected strain of house fly (Diptera: Muscidae) under laboratory selection, *J. Econ. Entomol.* **78**, 316 (1985).
 25. S. S. T. Lee and J. G. Scott, An improved method for preparation, stabilization and storage of house fly (Diptera: Muscidae) microsomes, *J. Econ. Entomol.* **82**, 1559 (1989).
 26. M. M. Bradford, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, *Analy. Biochem.* **72**, 248 (1976).
 27. N. E. Hopkins, M. K. Foroozesh, and W. L. Alworth, Suicide inhibitors of cytochrome P450 1A1 and P450 2B1, *Biochem. Pharmacol.* **44**, 787 (1992).
 28. M. Foroozesh, G. Primrose, Z. Guo, L. C. Bell, W. L. Alworth, and F. P. Guengerich, Aryl acetylenes as mechanism-based inhibitors of cytochrome P450-dependent monooxygenase enzymes, *Chem. Res. Toxicol.* **10**, 91 (1997).
 29. S. M. Strobel, G. D. Szklarz, Y. He, M. Foroozesh, W. L. Alworth, E. S. Roberts, P. F. Hollenberb, and J. R. Halpert, Identification of selective mechanism-based inactivators of cytochromes P450 2B4 and 2B5, and determination of the molecular basis for differential susceptibility, *J. Pharmacol. Exp. Ther.* **290**, 445 (1999).
 30. I. Szekely, L. Pap, and B. Bertok, MB-599, a new synergist in pest control, *Brighton Crop Protec. Conf. Pests Dis.* **1996** 473 (1996).
 31. S. S. T. Lee and J. G. Scott, Microsomal cytochrome P450 monooxygenases in the house fly (*Musca domestica* L.): Biochemical changes associated with insecticide resistance and phenobarbital induction, *Pestic. Biochem. Physiol.* **35**, 1 (1989).
 32. J. G. Scott, Cross-resistance to the biological insecticide abamectin in pyrethroid resistant house flies, *Pestic. Biochem. Physiol.* **34**, 27 (1989).
 33. D. J. Finney, Probit Analysis, University Press, Cambridge, 1971.
 34. M. Raymond, Presentation d'un programme Basic d'analyse log-probit pour micro-ordinateur, *Cah. ORSTROM Ser. Ent. Med. Parasitol.* **23**, 117 (1985).
 35. W. S. Abbott, A method of computing the effectiveness of an insecticide, *J. Econ. Entomol.* **18**, 265 (1925).
 36. A. Tozzi, A brief history of the development of piperonyl butoxide as an insecticide synergist, in "Piperonyl Butoxide: The Insecticide Synergist" (D. G. Jones, Ed.), p. 323, Academic Press, San Diego, 1998.
 37. M. Berenbaum and P. Feeny, Toxicity of angular furanocoumarins to swallowtail butterflies: Escalation in a coevolutionary arms race? *Science* **212**, 927 (1981).
 38. M. R. Berenbaum, Coumarins, in "Herbivores: Their Interactions with Secondary Plant Metabolites" (G. A. Rosenthal, and M. R. Berenbaum, Eds.), p. 221, Academic Press, New York, 1991.
 39. N. Liu, and J. G. Scott, Genetics of resistance to pyrethroid insecticides in the house fly, *Musca domestica*, *Pestic. Biochem. Physiol.* **52**, 116 (1995).
 40. T. Shimada, H. Yamazaki, M. Foroozesh, N. E. Hopkins, W. L. Alworth, and F. P. Guengerich, Selectivity of polycyclic inhibitors for human cytochromes P450 1A1, 1A2, and 1B1, *Chem. Res. Toxicol.* **11**, 1048 (1998).
 41. T. Kamataki, M. C. M. Lee Lin, D. H. Belcher, and R. A. Neal, Studies of the metabolism of parathion with an apparently homogenous preparation of rabbit liver cytochrome P-450, *Drug Metab. Dispos.* **4**, 180 (1976).
 42. L. E. Beebe, E. S. Roberts, L. W. Fornwald, P. F. Hollenberb, and W. L. Alworth, Mechanism-based inhibition of mouse P450_{2b-10} by selected arylalkynes, *Biochem. Pharmacol.* **52**, 1507 (1996).
 43. E. S. Roberts, N. E. Hopkins, M. Foroozesh, W. L. Alworth, J. R. Happort, and P. F. Hollenberb, Inactivation of cytochrome P450s 2B1, 2B4, 2B6, and 2B11 by arylalkynes, *Drug Metabol. Disp.* **25**, 1242 (1997).