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Synthesis of Pharmaceutical and Personal Care Products and Pesticide Metabolites of Environmental Concern

Isaiah Edwards

SYNTHESIS OF PHARMACEUTICAL AND PERSONAL CARE PRODUCTS AND PESTICIDE METABOLITES OF ENVIRONMENTAL CONCERN

By Isaiah Edwards

A thesis submitted to the faculty of The University of Mississippi in partial fulfillment of the requirements of the Sally McDonnell Barksdale Honors College.

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Approved by

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Advisor: Professor John Rimoldi

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Reader: Professor Courtney Roper

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Reader: Professor Kristine Willett

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ABSTRACT

ISAIAH EDWARDS: Synthesis of Pharmaceutical and Personal Care Products and Pesticide Metabolites of Environmental Concern

(Under the direction of Dr. John Rimoldi)

Human pollution of aquatic environments by introduction of pharmaceutical and personal care products (PPCPs) as well as agrichemicals presents a grave threat to the health of aquatic organisms. Many of these chemicals not only exhibit toxicity to the species of complex ecosystems at various levels but also undergo metabolic transformation into moieties that have different and sometimes more potent physiochemical profiles. PPCP and pesticides as well as their metabolites impact organisms via a number of different mechanisms, with the most serious arguably being endocrine disruption. Two emerging pollutants, gemfibrozil and bifenthrin, possess naturally occurring metabolites that were determined to warrant investigation for their toxicological and physiochemical properties. These metabolites exhibit markedly different pharmacological profiles from their parent compound. In order to facilitate the mission of investigating the unique properties of these metabolites, efficient synthesis protocols are required. The goal of this thesis was to perform and optimize the synthesis of both chlorinated and brominated adducts of gemfibrozil as well as the oxidative bifenthrin metabolite 4'-hydroxybifenthrin utilizing an organometallic oxidative approach. The halogenated gemfibrozil adducts were further used to support research that was ultimately submitted to the journal *Environmental Science and Technology*.

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LIST OF ABBREVIATIONS

PPCP	Pharmaceutical and personal care products
EPA	Environmental Protection Agency
ug	micrograms
L	liter
mL	milliliter
KCO_3	Potassium carbonate
LTA	Lead (IV) tetraacetate
TFA	Trifluoroacetic acid
KOH	Potassium hydroxide
EtOH	Ethanol
g	gram
DCM	Dichloromethane
mmol	millimole
KBr	Potassium bromide

Introduction and Background

The presence of pollutants from human sources in aquatic ecosystems presents a major threat to the health of aquatic species. Pharmaceuticals and personal care products (PPCP) are a vast heterogenous group of chemicals that "(are) used by individuals for personal health or cosmetic reasons or used by agribusiness to enhance growth or health of livestock" as defined by the EPA[1]. These chemicals and their metabolites readily disseminate into multiple compartments of aquatic ecosystem with varying affinity, where their presence drives detrimental changes to aquatic organisms[3]. In addition, many of these PPCPs and their metabolites exhibit significant ecotoxicity as they are transferred between various trophic levels. These chemicals disseminate into sediment, aqueous phase, and the organisms themselves with varying affinity. One of the major concerns of PPCPs and other agrochemicals in ecosystems is their tendency to serve as endocrine disruptors in aquatic species [3]. A major concern of the presence of pharmaceuticals and pesticides presence in aquatic environments is their ability to impact explicit physiological pathways at low doses, as was discussed by Fabbri[2]. Contrary to traditional pollutants, PPCPs are explicitly designed for effective physiological modulation at a specific, low dose, which causes morbidity when encountering conserved physiological systems in aquatic compartments. In addition to their potent physiological effects, persistence and bioaccumulation are also concerns, as bioaccumulation has a risk of moving up the food web to inadvertently and disproportionately affect apex organisms. An example of this was observed with experiments conducted on gemfibrozil, a common lipid controlling agent. Exposing gemfibrozil to goldfish over 14 days resulted in extensive bioaccumulation, with a bioaccumulation factor of 113[4]. Additionally,

continuous infusion of PPCPs into the environment from human wastewater, coupled with improper or incomplete removal, leads to their ubiquity in aquatic environments[5]. This continuous infusion into wastewater coupled with such widespread use and elimination of PPCPs in the world result in PPCPs being introduced in large quantities to the point where they are possibly ubiquitous worldwide (Figure 1).

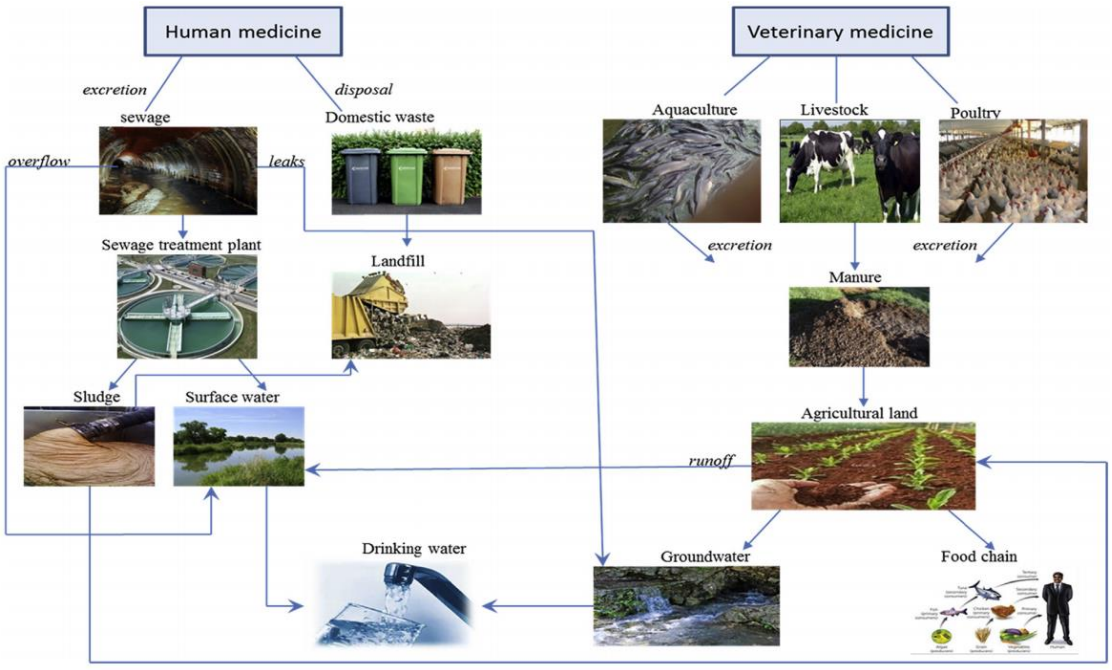


Figure I: Illustration of sources and destinations of PPCP pollutants[1]

Not only are PPCPs and agrochemicals themselves important to consider, biochemically and synthetically produced metabolites are also important to analyze. For instance, the extensive use of hypochlorite as a disinfection agent normally destroys or

renders inert all organics present in wastewater via its halogenation and oxidation potential. This has been illustrated by Bulloch[6] to occur in wastewater-consistent concentrations of hypochlorite and hypobromite (which is produced via halogen exchange) with gemfibrozil, producing 4'-chlorinated and brominated gemfibrozil analogs.

Gemfibrozil: Parent compound and chlorination adduct toxicology

Many PPCPs that organisms encounter exhibit various levels of toxicity in different via multiple mechanisms. For instance, gemfibrozil, when exposed aqueously to *Dreissena polymorpha* resulted in significantly levels of oxidative toxicity, reflected by altered levels of physiologically relevant biomarker concentrations[7]. At environmentally relevant concentrations of gemfibrozil, glutathione transferase and lipid peroxidation were highly elevated, both of which serve as biomarkers of oxidative damage. In addition, DNA damage was also observed after a period of 96 hours.

As mentioned earlier, endocrine disruption in aquatic species is one of the major concerns for PPCPs and their metabolites being introduced to natural biota, which imparts many health complications for affected species, including risk of tumors and disrupted development[3]. Gemfibrozil is a prime illustration of this. In addition to being bioaccumulative, environmentally relevant levels of gemfibrozil were illustrated to cause a 50% reduction in testosterone of treated goldfish[14]. In the interest of elucidating a mechanism, a 50% decrease in the steroid acute regulatory protein transcript in the exposed goldfish was also reported. This protein is integral in the transport of cholesterol in the mitochondria and is thereby a key component of steroidogenesis, which is integral in producing steroid sex hormones like testosterone. Additionally, Bulloch illustrated that

chlorogemfibrozil and bromogemfibrozil have enhanced anti-androgenicity in Japanese medaka, depleting levels of 11-ketoesterone and testosterone significantly, respectively, when compared to the parent compound of gemfibrozil. This represents a concerning trend of not only PPCPs themselves exerting acute toxicity in sensitives species, but also ambient processes, such as disinfection protocols, producing novel analogs that have extensively different biochemical and pharmacological profiles.

Bifenthrin and 4'-Hydroxybifenthrin toxicology

Another emerging contaminant that has exhibited signs of acute toxicity is bifenthrin, a widely used synthetic pyrethroid insecticide. Continuous oral infusion of bifenthrin into both goats[8] and rats[9] resulted in the observation of biomarkers indicating severe oxidative stress. Specifically, goats experienced a decrease in antioxidant parameters including glutathione peroxidase and free glutathione levels, and an increase in malondialdehyde levels, which was coupled with hematological alterations. Rats at the same relative dosage exhibited an increase in malondialdehyde levels as well as a decrease in catalase and free glutathione levels, which, just as in goats, reflects systemic oxidative damage. Thus, bifenthrin is illustrated to possess a strongly oxidative acute toxicity.

Bifenthrin also exhibits receptor mediated toxicity as well, which results in observations of neurotoxicity [0]. Bifenthrin operates as an excitotoxin and induces acute neurotoxicity in insect nerves by targeting voltage gated sodium channels, binding and delaying the channel's inactivation which eventually excites them[11]. This was canonically how bifenthrin's toxicity mechanism was believed to occur, but more recently a larger group of channels has been implicated in its binding and ultimate toxicity. By binding

channels that are homologous to those found in its insect targets, bifenthrin can produce observable neuronal derangements and behavioral changes in other biota as well. For instance, it was illustrated that exposing developing zebrafish larvae to environmentally relevant concentrations as low as 50 μg/L of bifenthrin resulted in presentation of muscular spasms in a dose-dependent manner[12]. This illustrates one possible mechanism by which bifenthrin can impart teratogenic changes to aquatic populations.

Bifenthrin also possesses potential as an endocrine disruptor, although the metabolites' estrogenicity or anti-estrogenicity are complex. *In vivo,* bifenthrin was anti-estrogenic in a chemical activated luciferase gene expression assay[13]. In contrast, metabolic conversion via P450 of bifenthrin to 4-hydroxy bifenthrin has been tentatively supported to drive enhanced estrogenicity *in vivo* at environmentally relevant concentrations[14]. When the 4'-hydroxy bifenthrin metabolite was exposed to fish, they had an increased expression of estrogen-mediated proteins, consistent with the *in vivo* metabolism inducing estrogenicity. Interestingly, the anti-estrogenic 4'-hydroxybifenthrin is the most commonly observed metabolite in fish when they are exposed to bifenthrin[15]. This could illustrate the levels of differential toxicities and complex interactions that these metabolites have in aquatic ecosystems. From the information above, it is clear that bifenthrin and its subsequent biotransformation products have complex interactions with aquatic organisms which result demonstrable changes to these organisms' physiologies.

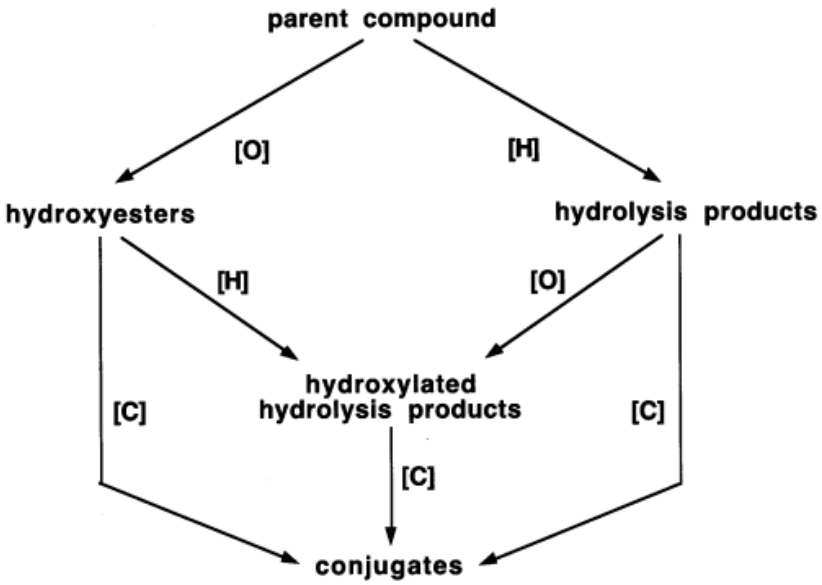


Figure II: Generalized pathway for metabolism of pyrethroids in mammals by Oxidation[O], Hydrolysis [H], and Conjugation[C] reactions[11]

Bifenthrin and Gemfibrozil: Prevalence and Acute Threats to Aquatic Ecosystems

Gemfibrozil and bifenthrin carry both latent transformation potential by metabolism or wastewater processes and significant risk to organisms exposed to either these metabolites or their parent precursor. Missing from the previous discussion is a realistic look at their prevalence within the environment and data supporting their impact on these environments.

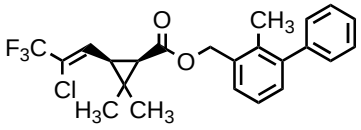
Gemfibrozil presence in wastewater treatment has been studied and wastewater influent and effluent concentrations were quantified[16]. Influent concentration ranges were from 3.47 to 63.8 μg/L, and effluent concentrations were 0.08 to 19.4 μg/L, with the latter containing concentrations that have been experimentally illustrated to be biologically

active. Dissipation half-life of gemfibrozil into sandy and silty loam soils were on the timeframe of 17-21 days. Utilizing a river water microcosm experiment with treatment of 100 μg/L concentrations of gemfibrozil, the persistence of gemfibrozil in sediment was also quite significant, with the half-life being 70 days. This, in addition to its appreciable effluent concentration range, support gemfibrozil's ubiquity and pseudo-persistence. In terms of sedimentary toxicity, Andrzejczyk et. al. were able to illustrate bioaccumulation of gemfibrozil as well as chloro- and bromogemfibrozil when exposing *Neanthes arenaceodentata* to sediment contaminated with chlorogemfibrozil and bromogemfibrozil[17]. While the risk for acute toxicity was determined to be low, there was determined to be a risk of bioaccumulation resulting in passage up through the food web to apex species, whose lifelong exposure exacerbates low but chronic exposure.

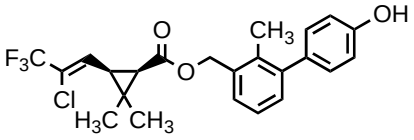
Bifenthrin exposure to the environment and waterways is thought to follow a slightly different path than gemfibrozil; it is believed to be a product of urban and suburban runoff. Bifenthrin is a major contaminant of urban and heavy agricultural areas, likely associated with its ubiquitous commercial and agricultural use. In fact, heavy urbanization was spatially linked to an increase in highly toxic concentrations of pyrethroid pesticides, including bifenthrin, in Guangzhou College City[18]. In Australia, pyrethroid compounds, mainly bifenthrin and permethrin, inhabit sediment concentrations that impact aquatic organisms' fitness[19]. Dandenong Creek was analyzed for its organic and metallic pollution content. Impaired fitness of *C. tepperi* populations as well as depleted aquatic diversity were observed where sediment concentrations of organic pollutants, primarily containing pyrethroid pesticide residues, were dominant. In contrast to correlational studies, bifenthrin's potential for ecotoxicity has also been

illustrated experimentally by Rogers et.al.[20] Using mesocosm experimentation, bifenthrin exposure to small stream communities reduced larval macroinvertebrate abundance and diversity at nanogram concentration scale. Furthermore, a trophic cascade was observed as macroinvertebrate scrappers were depleted that lead to an increase in periphyton populations. This experiment highlights the acute and unforeseen risks even nanogram scale pollution of bifenthrin can effect on complex aquatic ecosystems.

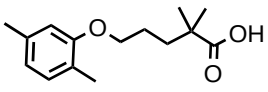
With the extent and gravity that problems of PPCP and agrochemical pollution present to nature, especially aquatic ecosystems, the study of these systems becomes a necessity. In studying the effects and extent of emerging pollutants and their transformation products, the ability to readily and reliably synthesize these moieties becomes of utmost importance to facilitate effective investigation of these compounds, their extent, and their potential dangers. The thesis research described focuses on the synthesis and characterization of the metabolites of two well-characterized pollutants, gemfibrozil and bifenthrin, to facilitate investigation of their toxicological and physiochemical properties.



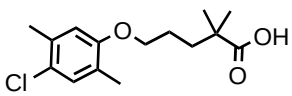
Bifenthrin



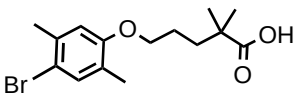
4'-Hydroxybifenthrin



Gemfibrozil



3-Chlorogemfibrozil



3-Bromogemfibrozil

Figure III: Structures of parent compounds and metabolites of common PPCP and agrochemical pollutants investigated

Synthesis of 4'-hydroxybifenthrin

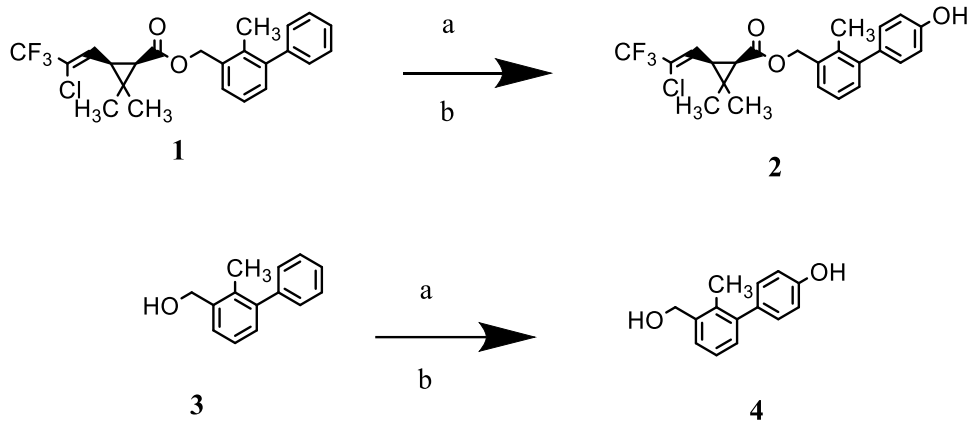


Figure IV: Synthesis of 4'-hydroxybifenthrin (5) and 4'-hydroxy BP alcohol (4).
Experimental Conditions: a.) LTA, TFA, room temperature. b.) 10% KCO_3, **2**, (1.3%); **4** (5.5%)

Results and Discussion

Synthesis of 4'-hydroxybifenthrin (2) was based on the protocol utilized by Zhang and Scott[21]. Electrophilic substitution of bifenthrin (1) using an organometallic reagent, Lead (IV) tetraacetate (LTA) in trifluoroacetic acid (TFA), resulted in acylation of bifenthrin at the 3- and 4'- positions followed by selective hydrolysis with 10% potassium carbonate solution. Oxidation of the 4'- position, producing 4'-hydroxybifenthrin (**2)**, was preferred. In an attempt to optimize the yields of the reported synthesis, the ester present in bifenthrin and the products was preemptively hydrolyzed, and the biphenyl alcohol (**3)** fragment was subjected to the same reaction conditions as was employed in oxidizing the whole molecule in order to discern if low yields were an effect of inadvertent hydrolysis or of steric hinderance of the oxidative process. Hydrolysis of bifenthrin followed the procedure of Banasiak[22], utilizing an 6.7% KOH/EtOH mixture under reflux. Due to the cost prohibitive nature of purchasing adequate bifenthrin from commercial vendors, bifenthrin was purified from a commercially available pesticide solution. Bifenthrin was

isolated from the carrier solution xylene via vacuum filtration, and this highly purified bifenthrin was used in all reactions.

Synthesis of 4'-hydroxybifenthrin proved more challenging. The yields reported by Zhang were 3-4% for 4'-hydroxybifenthrin utilizing LTA/TFA oxidation protocols. Both reactions performed on both 1 gram and 5 gram scale produced reaction yields of 1.3% in both cases. Over-oxidation and possibly premature hydrolysis in the highly acidic reaction conditions were speculated to have been the cause of this low yield, as was evident on heavy spotting at low Rf values on reaction TLCs, suggesting highly polar moieties present in the reaction mixture. To ascertain whether this was the case, the hydrolyzed fragment of bifenthrin, (2-methyl-[1,1'-biphenyl]-3-yl)methyl alcohol (BP alcohol; **3)** was subjected to the same reaction conditions. The yield from this experiment for 4'-hydroxy BP alcohol product (**4)** was 5.5%. In addition to the apparent increase in yield, separation was also far easier with this product mixture than with the complex oxidative and hydrolytic product mixture of whole molecule reaction. Coupling of thus obtained 4'-hydroxy BP alcohol product with the other acid fragment of the hydrolysis product from bifenthrin, (1R,3R)-3-((Z)-2-chloro-3,3,3-trifluoroprop-1-en-1-yl)-2,2-dimethylcyclopropane-1-carboxylic acid (TFP acid) could easily be achieved with a variety of esterification methods, and in addition this experiment illustrates a demonstrable improvement in reaction yields.

Materials and methods

General Methods

All reactions were performed using commercially available materials. Every reaction was performed under inert atmospheric conditions. Each reaction product was analyzed using thin layer chromatography (TLC) to monitor reaction completion and product mixtures. Phosphomolybdic acid was used to stain TLC plates. Low resolution mass spectroscopy was used to confirm product presence in the product mixtures. For all Nuclear Magnetic Resonance (NMR) data, a Bruker 400 MHz Avance NMR spectrometer was used, and all raw data was processed with MestReNova software.

Experimental:

Isolation of bifenthrin from commercially available preparation

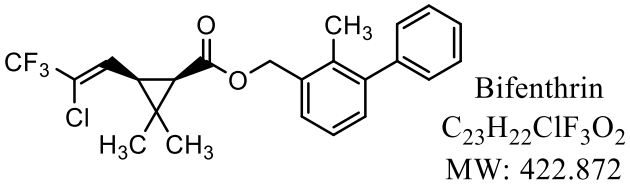


Figure V: Bifenthrin structure

100 mL of a commercially available bifenthrin pesticide formulation (Bifen XTS©) was subjected to vacuum distillation at temperatures of 70-80 ^{0}C. From this, 65 mL of xylene was eluted and 32 G of bifenthrin remained as a colorless, viscous, oily residue. ^{1}H NMR (500 MHz, Acetone-d_6) δ 7.46 (dd, J = 8.1, 6.7 Hz, 2H), 7.43 – 7.36 (m, 2H), 7.36 – 7.31 (m, 2H), 7.28 (s, 1H), 7.23 (d, J = 1.5 Hz, 1H), 5.26 (d, J = 6.2 Hz, 2H), 2.37 – 2.26 (m, 2H), 2.23 (s, 3H), 1.35 (d, J = 13.2 Hz, 9H), 0.89 (s, 1H). ^{1}H NMR (400 MHz, Chloroform-d) δ 7.57 – 7.23 (m, 8H), 7.09 – 6.95 (m, 1H), 5.35 – 5.15 (m, 2H), 2.27 (s, 3H), 2.25 – 2.20 (m, 1H), 2.11 (d, J = 8.3 Hz, 1H), 1.35 (d, J = 7.5 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.17, 143.02, 141.82, 134.46, 134.26, 130.41, 130.12, 130.08, 129.35,

128.41, 128.13, 126.94, 125.65, 77.40, 77.09, 76.77, 65.40, 32.94, 30.97, 28.76, 28.37, 16.20, 14.98, 14.12. LCMS Exact mass calculated for $C_{23}H_{22}ClF_3O_2Na$ (MNa^+) 445.12, found: 445.17.

Hydrolysis of bifenthrin to BP-alcohol and TFP acid

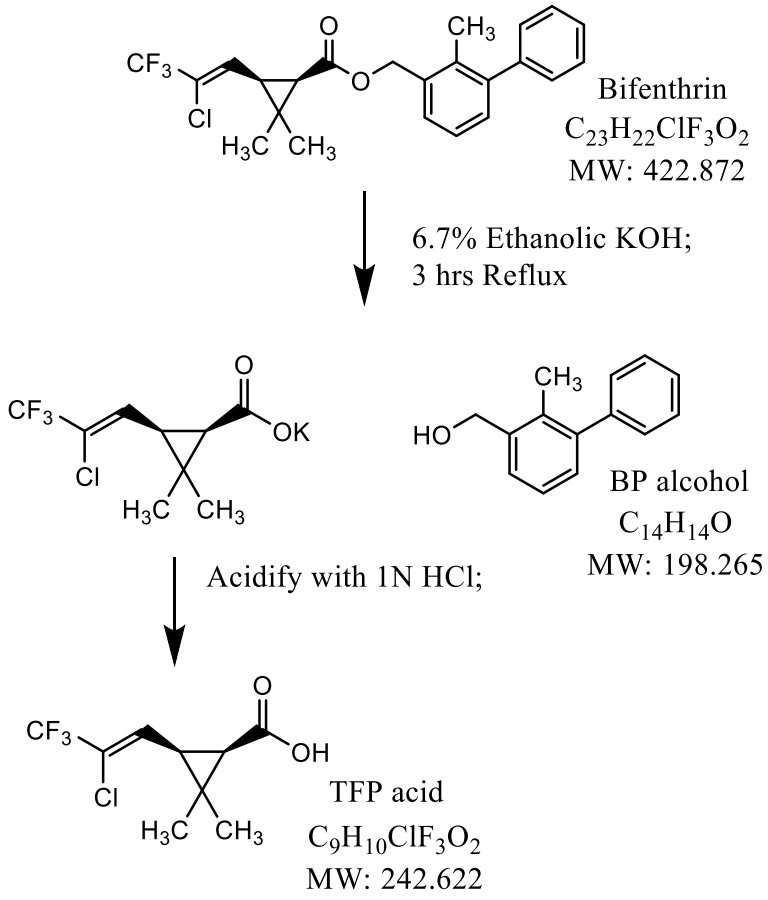


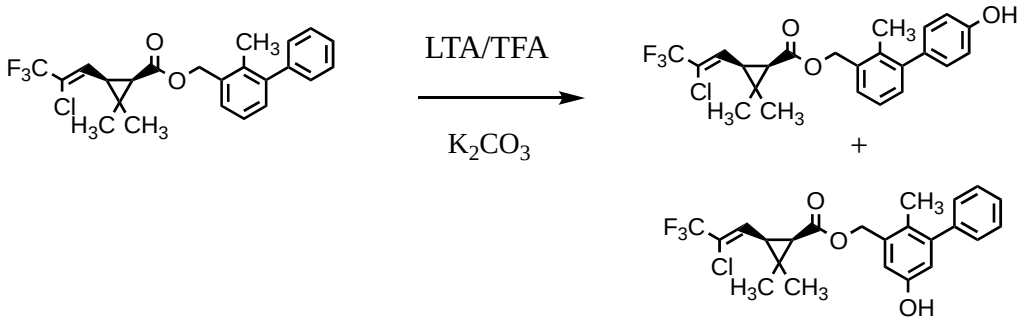
Figure VI: Bifenthrin hydrolysis scheme

Bifenthrin was dissolved into 6.7% Ethanolic KOH. This solution was refluxed for 3 hours, and the ethanol was evaporated. The products were dissolved into water and extracted with DCM, and this fraction contained the BP alcohol (**3)** moiety. ^{1}H NMR (400

MHz, Chloroform-*d*) δ 7.85 – 6.63 (m, 9H), 4.63 (s, 2H), 2.11 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 142.80, 142.07, 139.28, 133.51, 129.51, 129.41, 128.08, 126.80, 126.70, 125.59, 77.41, 77.09, 76.77, 63.93, 15.89.

The aqueous portion was acidified with 1N dilute HCL, which was then extracted with DMC 3 times. This organic fraction was washed with water and dried with anhydrous sodium sulfate, filtered, and evaporated to give TFP acid. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 9.90 (s, 1H), 4.14 (q, *J* = 7.1 Hz, 1H), 3.63 – 3.40 (m, 1H), 2.64 – 2.15 (m, 2H), 1.41 – 1.07 (m, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 198.02, 176.30, 166.92, 77.37, 77.05, 76.74, 61.57, 50.97, 41.53, 34.83, 34.29, 20.48, 19.82, 14.11.

Bifenthrin oxidation in LTA/TFA solution



By-product

1 g of bifenthrin (**1)** (2.37 mmol) was added into a chilled and stirred solution of 1.6 grams (2.4 mmol) of lead (IV) tetraacetate (LTA) and 6 mL of trifluoroacetic acid. Immediately after addition to the reaction vessel, a dark brown coloration was observed which eventually dissipated. The reaction was allowed to continue at room temperature for 96 hours, at which point it was quenched by the addition of 10% potassium carbonate solution.

The reaction mixture was extracted thrice with ethyl acetate, which was dried with anhydrous sodium sulfate and evaporated to leave a sticky brownish yellow residue. This was subjected to purification using silica gel column chromatography using 20% ethyl acetate/hexanes as an eluent solvent system. This purification step resulted in isolation of seven fractions (A to G) as shown in the Thin layer chromatography (TLC) picture below, which also includes crude product mixture shown in the middle. Proposed structures and identities are listed in Figure X.

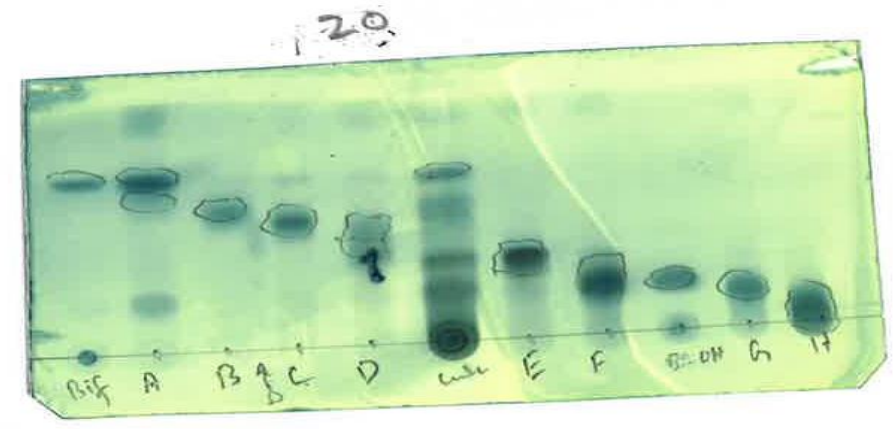


Figure VIII: TLC of Bifenthrin oxidation

Fraction-E (46 mg) was observed to be the product of interest showing a mixture of two structurally isomeric hydroxy-bifenthrins. That was further purified by repeating the silica gel purification to yield 13 mg of 4'-hydroxybifenthrin (**2)** (1.3% yield) and 22 mg of 5-hydroxybifenthrin (2.2% yield) as a by-product.

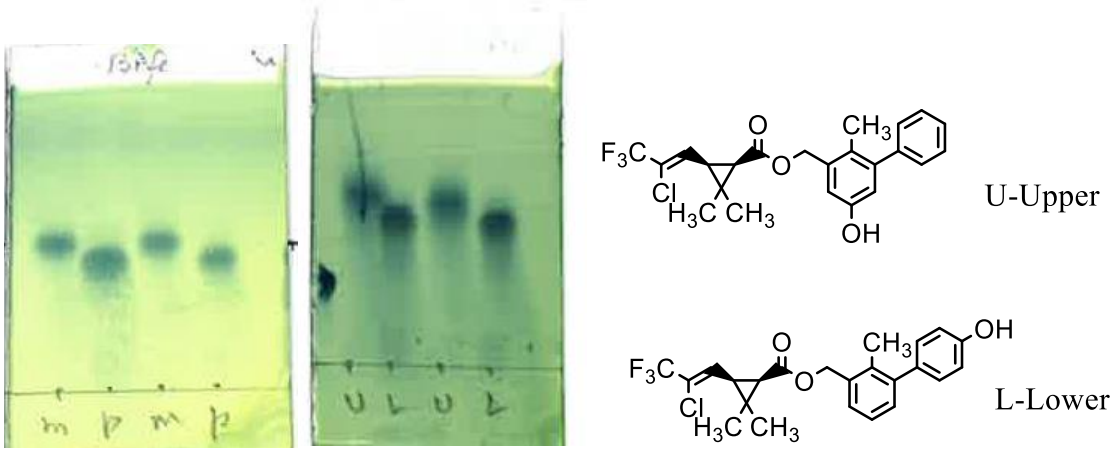


Figure IX: Differential TLC of hydroxybifenthrin isomers

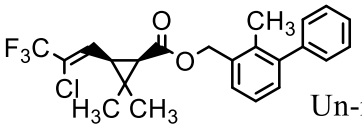
4'-Hydroxybifenthrin (L-Lower/Para-isomer): ^{1}H NMR (500 MHz, Acetone-d_6) δ 7.48 – 7.09 (m, 5H), 6.97 – 6.90 (m, 2H), 5.59 (s, 1H), 5.24 (d, J = 6.6 Hz, 2H), 2.35 – 2.22 (m, 5H), 1.34 (dd, J = 16.1, 3.3 Hz, 6H). ^{13}C NMR (126 MHz, Acetone) δ 206.53, 206.38, 170.77, 157.42, 143.59, 135.54, 135.13, 133.65, 132.48, 131.19, 130.98, 128.68, 126.30, 115.78, 65.95, 33.65, 31.26, 30.39, 30.30, 30.24, 30.15, 30.09, 29.99, 29.93, 29.84, 29.84, 29.78, 29.69, 29.63, 29.53, 29.47, 29.38, 27.95, 16.31, 15.13. LCMS Exact mass calculated for $C_{23}H_{22}ClF_3O_3Na$ (MNa^+) 461.11, found: 461.12.

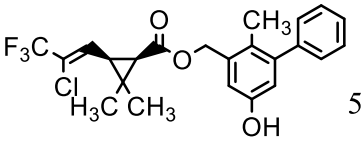
5-Hydroxybifenthrin (U-Upper/Meta-isomer): ^{1}H NMR (500 MHz, Acetone-d_6) δ 7.50 – 7.25 (m, 3H), 7.12 (d, J = 9.3 Hz, 2H), 6.82 (dd, J = 96.0, 2.7 Hz, 2H), 5.31 – 5.06 (m, 2H), 2.39 – 2.14 (m, 3H), 2.10 (s, 2H), 1.34 (dd, J = 17.5, 6.1 Hz, 6H). ^{13}C NMR (126 MHz, Acetone) δ 205.29, 169.88, 154.89, 143.83, 142.07, 135.95, 131.60, 131.56, 129.07, 128.09, 126.85, 124.40, 116.52, 115.30, 64.93, 32.82, 30.44, 29.42, 29.29, 29.26, 29.18, 29.11, 29.02, 28.95, 28.87, 28.80, 28.72, 28.65, 28.64, 28.49, 27.14, 14.49, 14.31. LCMS Exact mass calculated for $C_{23}H_{22}ClF_3O_3Na$ (MNa^+) 461.11, found: 461.12.

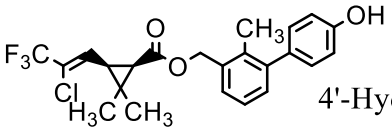
The remainder of the fractions were identified as hydrolyzed by-products - BP-alcohol (90 mg), 5-Hydroxy- BP-alcohol (35 mg), 4'-Hydroxy-BP-alcohol (12 mg), TFP-acid (260 mg) along with considerable amounts of un-reacted staring material i.e. Bifenthrin (480 mg).

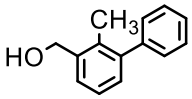
This procedure was scaled up under identical conditions as described above with 5 grams of bifenthrin (11.8 mmol) being added to 7.52 g (11.4 mmol) of LTA in 30 mL of trifluoroacetic acid to yield 65 mg of 4'-hydroxybifenthrin (**2)** (1.3% yield) and 100 mg of 5-hydroxybifenthrin (2.2% yield) confirming the reproducibility of the product distribution under identical reaction conditions.

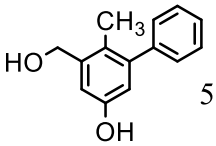
Thin layer chromatography pattern of Bifenthrin and the resultant product fractions isolated:

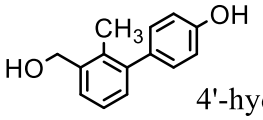
A  Un-reacted Starting Material (Bifenthrin)

E-Upper  5-Hydroxybifenthrin

E-Lower  4'-Hydroxybifenthrin

F-Upper  BP-alcohol

F-Lower  5-hydroxy-BP-alcohol

G  4'-hydroxy-BP-alcohol

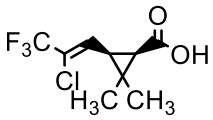
H  TFP-acid

Figure X: Proposed products formed from LTA oxidation corresponding to Figure VIII

BP alcohol oxidation using LTA/TFA solution

0.5 grams of BP alcohol (**3**) was added to a solution of 2.8 grams of LTA in 11.5 mL of trifluoroacetic acid. The reaction mixture turned a royal purple, followed by a medium green after 10 minutes of reaction. The reaction was allowed to continue for 72 hours, at which point it was quenched with 10% Potassium carbonate solution. The quenched reaction mixture was extracted thrice with ethyl acetate, which was dried with anhydrous sodium sulfate and evaporated. The whitish powder that remained was subjected to silica column chromatography at 20% ethyl acetate/hexanes, which yielded 30 mg of 4'-hydroxy BP alcohol (**4**) (5.5% yield).

Preparation and characterization of chlorinated and brominated Gemfibrozil analogs.

Results and Discussion

Concerning gemfibrozil, the transformation compounds of growing interest, which were highlighted to be highly endocrine disruptive, that we sought to synthesize were 3-chlorogemfibrozil and 3-bromogemfibrozil. To reflect wastewater conditions where these compounds would have ultimately originated via halogenation of the parent compound gemfibrozil, a simple hypohalite chlorinating agent was utilized in the reaction with gemfibrozil: household bleach. Utilizing 8.3% Sodium hypochlorite solution in methanol, gemfibrozil was chlorinated to 3-chlorogemfirbrozil**.** For the brominated adduct, a simple reaction was performed on the sodium hypochlorite by adding potassium bromide to produce sodium hypobromite, which ultimately drove the bromination reaction to produce 3-bromogemfibrozil. This synthesis procedure was adapted from Bulloch et. al. with slight modifications in the pursuit of optimization[6]. This modification was the employment of methanol as the reaction solvent instead of deionized water.

Gemfibrozil halogenation reactions were remarkably efficient and quite easy to carry out and isolate the products. Both the bromination and chlorination protocols resulted in 75% yield, which is very striking considering the relatively low concentration of halogenating agent used (8.3% sodium hypochlorite and presumably an equimolar amount

of hypobromous acid in the brominating reaction). Both the yield and timeframe of these halogenation reactions resulted in a very favorable and efficient protocol.

Materials and methods

General Methods

All reactions were performed using commercially available materials. Every reaction was performed under inert atmospheric conditions. Each reaction product was analyzed using thin layer chromatography (TLC) to monitor reaction completion and product mixtures. Phosphomolybdic acid was used to stain TLC plates. Low resolution mass spectroscopy was used to confirm product presence in the product mixtures. For all Nuclear Magnetic Resonance (NMR) data, a Bruker 400 MHz Avance NMR spectrometer was used, and all raw data was processed with MestReNova software.

Experimental:

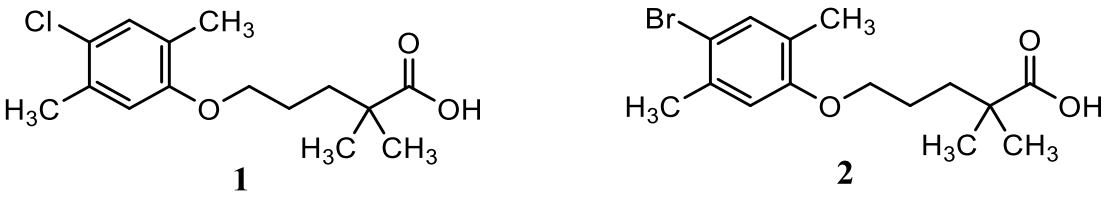


Figure XI: Halogenation products of gemfibrozil

These halogenated analogs were prepared by following the method of Bulloch et al., with slight modification. Gemfibrozil (251 mg; 1mmol) was dissolved in about 12 ml solvent mixture comprising methanol (10 ml) and water (2 ml), and to this solution, a fresh

liquid bleach of sodium hypochlorite (Clorox® Germicidal Bleach; 8.3%; 800 mg; 10 mmol; 10 ml) was added dropwise while stirring the contents. Then, after two hours of stirring at room temperature, the reaction was quenched with aqueous sodium thiosulfate solution (11 mmol; 1.7 g/ 10 ml), contents were extracted twice with ethyl acetate (20 ml x2), organic layer was collected, dried under anhydrous sodium sulfate, filtered, and concentrated under rotavac to get a white residue which was further recrystallized in diethyl ether to afford chlorogemfibrozil (**1**; 212 mg; 75% yield).

^{1}H NMR (400 MHz, methanol-d_4) δ 7.02 (s, 1H), 6.75 (s, 1H), 3.91 (t, J = 6.4 Hz, 2H), 2.28 (s, 3H), 2.12 (s, 3H), 1.77 (td, J = 6.5, 3.5 Hz, 2H), 1.69 – 1.52 (m, 2H), 1.15 (s, 6H). ^{13}C NMR (101 MHz, MeOD) δ 185.00, 155.82, 133.36, 129.84, 125.62, 124.09, 113.21, 68.69, 48.24, 48.03, 47.82, 47.60, 47.39, 47.35, 47.18, 46.97, 42.77, 37.68, 25.47, 25.32, 18.63, 14.36. ESI-MS calculated for $C_{15}H_{21}ClO_3$: M+Na/z 307.11; Found: M+Na/z 307.11.

The brominated analog was prepared following the procedure as described above but by using sodium hypobromite solution which was extemporaneously prepared by treating KBr (13 mmol; 1.55 g) to sodium hypochlorite (Clorox® Germicidal Bleach; 8.3%; 800 mg; 10 mmol; 10 ml). The slightly yellow sticky residue thus obtained was further recrystallized in diethyl ether to afford bromogemfibrozil (**2**; 246 mg; 75% yield).

^{1}H NMR (400 MHz, Methanol-d_4) δ 7.19 (s, 1H), 6.75 (s, 1H), 3.90 (t, J = 6.4 Hz, 2H), 2.30 (s, 3H), 2.11 (s, 3H), 1.77 (dd, J = 6.6, 3.7 Hz, 2H), 1.71 – 1.59 (m, 2H), 1.17 (s, 6H). ^{13}C NMR (101 MHz, MeOD) δ 183.80, 156.41, 135.31, 133.00, 125.96, 113.76, 113.19, 68.45, 48.50, 48.28, 48.07, 47.86, 47.65, 47.43, 47.22, 47.01, 42.40, 37.44, 25.20, 25.17, 21.57, 14.32. ESI-MS calculated for $C_{15}H_{21}BrO_3$: M-H/z 328.07; Found: M-H/z 328.04.

Conclusion

As stated earlier, the ready and efficient synthesis of the metabolites of emerging pollutants is integral in allowing for the study and characterization of their novel and potentially detrimental physiochemical properties. These syntheses provide standards and reagents for further experimentation as well as provide insights into the processes by which these metabolites arise, especially those that occur ex vivo as is the case with the halogenation adducts. For bifenthrin, this thesis highlighted the inefficiency of whole molecule oxidation when compared to yields of hydrolyzed oxidation, which were 1.3% versus 5.5%, marking a potentially more efficient, though more contrived, route of synthesis. Additionally, coupling of the oxidized BP alcohol to TFP acid still remains, which would undoubtedly decrease the yield further; however, this approach remains viable assuming a reasonably efficient coupling protocol exists. The results also elucidate the complex mixture that whole molecule organometallic oxidation produces, which is useful for future chemists to more reliably recreate this experiment and ultimately synthesize the molecule independently.

The gemfibrozil halogenation reaction proved to be extremely efficient and simple in terms of procedure and reagents, producing appreciable yields with almost exclusively cheap commercially available reagents. In addition to the contributions towards the chemistry, the halogenated adducts themselves were utilized to support the research of Dr. Schlenk at the University of California, Riverside (Nicolette et al.[17]), which was ultimately

submitted and accepted to the journal *Environmental Science and Technology*. Ultimately, by exploring and optimizing the synthesis of these metabolites and those like it, further research by multidisciplinary teams into the novel impacts of these emerging pollutants' metabolites on the environment and the organisms therein is facilitated.

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