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Ring-Fluorinated Analog of Methyl Eugenol: Attractiveness to and Metabolism in the Oriental Fruit Fly, *Bactrocera Dorsalis* (Hendel)

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Abstract Oriental fruit fly, *Bactrocera dorsalis* (Hendel), males are highly attracted to the natural phenylpropanoid methyl eugenol (ME). They compulsively feed on ME and metabolize it to ring and side-chain hydroxylated compounds that have both pheromonal and allomonal functions. Side-chain metabolic activation of ME leading to (*E*)-coniferyl alcohol has long been recognized as a primary reason for hepatocarcinogenicity of this compound in rodents. Earlier, we demonstrated that introduction of a fluorine atom at the terminal carbon of the ME side chain significantly depressed metabolism and specifically reduced formation of coniferyl alcohol but had little effect on field attractiveness to *B. dorsalis*. In the current paper, we demonstrate that fluorination of ME at the 4 position of the aromatic ring blocks metabolic ring-hydroxylation but overall enhances side-chain metabolism by increasing production of fluorinated (*E*)-coniferyl alcohol. In laboratory experiments, oriental fruit fly males were attracted to and readily consumed 1,2-dimethoxy-4-fluoro-5-(2-propenyl)benzene (I) at rates similar to ME but metabolized it faster. Flies that consumed the fluorine analog were as healthy post feeding as ones fed on methyl eugenol. In field trials, the fluorine analog I was ~50% less attractive to male *B. dorsalis* than ME.

Keywords Oriental fruit fly · *Bactrocera dorsalis* (Hendel) · Methyl eugenol · 1,2-dimethoxy-4-fluoro-5-(2-propenyl) benzene · Metabolism · Field attraction

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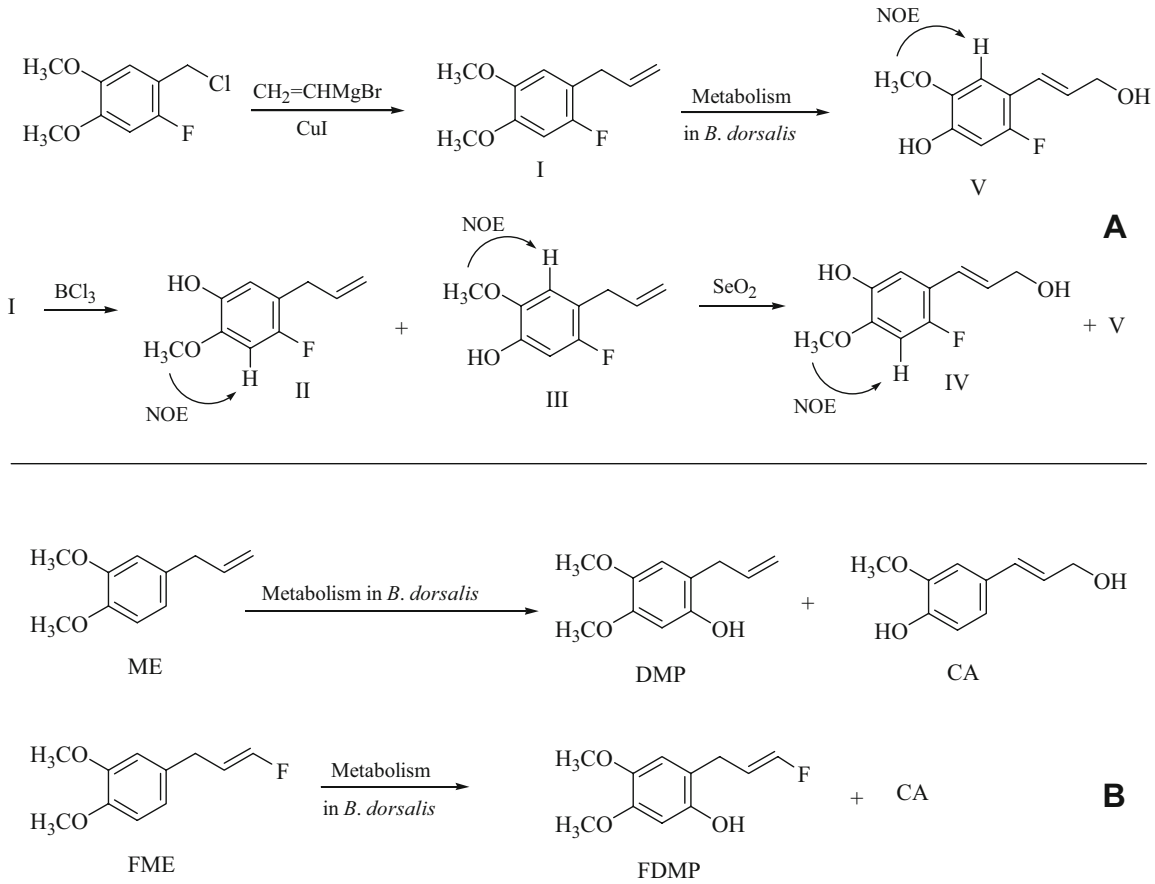
Introduction

The oriental fruit fly, *Bactrocera dorsalis* (Hendel), is a widespread pest of a broad range of tropical, subtropical, and temperate host plants (White and Elson-Harris 1992; US Department of Agriculture 1983). Males of *B. dorsalis* are strongly attracted to and compulsively feed on methyl eugenol (ME, Scheme 1; Steiner 1952), a commonly occurring plant phenylpropanoid (Metcalf 1990). The use of ME in traps and killing stations is an integral part of successful detection, control, and eradication strategies for the oriental fruit fly populations worldwide (Steiner et al. 1965; Koyama et al. 1984). The attraction to and consumption of ME by *B. dorsalis* and sibling species of the *dorsalis* complex, *B. papayae* and *B. carambolae*, have ecological significance in the fly's communication system. Mature



Scheme 1 **A** Synthesis and metabolism of the ring-fluorinated analog I in *B. dorsalis*, and chemical transformation of I to metabolite V. **B** Metabolism of methyl eugenol (*ME*) and the side-chain fluorinated analog of ME (*FME*) in *B. dorsalis*.

males, after feeding on ME, metabolize it primarily to 2-(2-propenyl)-4,5-dimethoxyphenol (DMP) and (*E*)-coniferyl alcohol (CA), which display pheromonal and allomonal properties (Nishida et al. 1988; Tan and Nishida 1996).

A potential problem to the continued use of ME in area-wide pest management programs is the decision by the National Toxicology Program, US Department of Health and Human Services to classify this compound as a carcinogen (National Toxicology Program 1998, 2002). Metabolic activation of ME and production of 1′-hydroxy metabolites through enzymatic side-chain hydroxylation are thought to be primarily responsible for hepatotoxicity and carcinogenicity of ME (Smith et al. 2002). Since early warnings about the carcinogenicity of ME (Miller et al. 1983; Schiestl et al. 1989; Sekizawa and Shibamoto 1982), a number of compounds have been evaluated as attractants for *B. dorsalis* (Metcalf et al. 1975; Mitchell et al. 1985; Khrimian et al. 1993, 1994; Liquido et al. 1998). One promising replacement for ME is a fluorine analog, (*E*)-1,2-dimethoxy-4-(3-fluoro-2-propenyl)benzene (FME, Scheme 1), which in limited field tests was as attractive to *B. dorsalis* as methyl eugenol (Khrimian et al. 1994). It is also more persistent than ME and, remarkably, about twice as active as the corresponding *Z* isomer (Khrimian et al. 1994; Liquido et al. 1998). This compound also displays a reduced propensity to oxidative biotransformations, specifically a side-chain hydroxylation in *B. dorsalis* (Khrimian et al. 2006), which is linked to the hepatotoxicity and carcinogenicity of ME (Smith et al. 2002). While the mechanism is still unclear, FME showed reduced toxicity and reduced recombinagenicity in yeast deletion assays compared to ME (Brennan et al. 1996). In the current paper, we present the results of our studies on behavioral bioassays and metabolism of yet another fluorinated ME analog, 1,2-dimethoxy-4-fluoro-5-(2-propenyl)benzene (I, Scheme 1). Of particular interest is how the fluorine introduction at a key metabolic hydroxylation site of ME would affect the overall biotransformation in oriental fruit fly. In addition, we were interested to quantify the attractiveness of compound I relative to its analogs ME and DMP, which also was highly attractive to *B. dorsalis* males (Nishida et al. 1988). There is ample information in the literature demonstrating that replacement of a hydroxy group with fluorine in the biologically active compounds may be successful (Welch 1987, 1991). In

addition, we expected such fluorine substitution to render an improved volatility compared to DMP, which may be beneficial for a fruit fly attractant.

Methods and Materials

Analytical Procedures ^{1}H NMR spectrum of I was recorded at 300 MHz in $CDCl_3$ with TMS as an internal standard on a Bruker QE-300 spectrometer. ^{1}H NMR spectra of other compounds were obtained at 500 MHz on a Bruker DRX-500 using TMS as an internal standard. Chemical shifts are reported in δ units, and coupling constants in Hz. GC analyses were performed on a Shimadzu 17A (Shimadzu Scientific Instruments, Inc., Columbia, MD, USA) gas chromatograph equipped with a flame ionization detector, an auto sampler AOC-20s and auto injector AOC-20i, and an HP-5 capillary column (30 m×0.25 mm×0.25 μm film). Hydrogen was used as carrier gas at 1 ml/min. Column temperature was maintained at 100°C for 2 min and then raised to 260°C at 10°C/min. Electron-ionization (EI) mass spectra were obtained at 70 eV with an Agilent Technologies 5973 mass selective detector interfaced with a 6890 N GC system equipped with a 30 m×0.25 mm i.d.×0.25 μm film HP-5MS column. Column temperature was maintained at 80°C for 1 min and then raised to 270°C at 10°C/min. Helium was used as carrier gas at 1 ml/min. TLC analyses were conducted on Whatman AL SIL G/UV plates using hexanes/ethyl acetate as a mobile phase with a 20% ethanol solution of phosphomolybdic acid and/or UV used for visualization of spots. Flash chromatography was carried out with 230–400 mesh silica gel (Fisher Scientific, Fair Lawn, NJ, USA).

Chemicals All reagents and solvents were purchased from Aldrich Chemical Co. unless otherwise specified.[1] Methylene chloride and hexamethylphosphoramide (HMPA; caution, it is a carcinogen!) were distilled from calcium hydride, and tetrahydrofuran (THF) was dried by distillation from sodium benzophenone-ketyl. Methyl eugenol metabolites, CA and DMP, were prepared from ferulic acid (Quideau and Ralph 1992) and 3,4-dimethoxyphenol (Benbow and Katoch-Rouse 2001), respectively. FME was prepared as described (Khrimian et al. 2006). 1-Chloromethyl-2-fluoro-4,5-dimethoxybenzene was prepared following a procedure of Furlano and Kirk (1986). The crude chloride was used in a copper-catalyzed Grignard coupling without further purification as described (Khrimian et al. 1993).

[1] Mention of commercial products does not constitute an endorsement by USDA.

1-Fluoro-4,5-dimethoxy-2-(2-propenyl)benzene (I) A solution of 1-chloromethyl-2-fluoro-4,5-dimethoxybenzene (1.62 g, 7.92 mmol) in dry THF (8 ml) was introduced under N_2 to a three-neck flask and cooled to −10°C. A solution of copper(I) iodide (150 mg, 0.79 mmol) in dry HMPA (6 ml) was added at once, followed by vinylmagnesium bromide (11.88 mmol; 11.88 ml of 1.0 M in THF) delivered slowly at −5–(−10)°C. The mixture was stirred at that temperature range for 30 min, then slowly warmed to 25°C over ~4 hr. The mixture was poured into a saturated ammonium chloride solution and extracted with ether (3×50 ml). The combined organic extracts were washed three times with saturated NH_4Cl and dried with Na_2SO_4. After evaporation of the solvent, the remainder was distilled in vacuum at 62–64°C/0.3 mm Hg to give I (800 mg, 52%) of 96% purity. Flash chromatography of this material on SiO_2 with hexanes/ethyl acetate, 8:1, gave I (600 mg) of 99% purity. ^{1}H NMR: 3.34 (dd, 2H, H-1′, J= 6.3, 1.2), 3.84 (s, 6H, OCH_3), 5.07 (m, 2H, H-3′), 5.94 (m, 1H, H-2′), 6.62 (d, $^3J_{HF}$=10.8, H-3), 6.65 (d, $^4J_{HF}$=7.5, H-6). GC-MS (EI): 196 (M^+, 100%), 181 (85), 169 (49), 165 (75), 125 (75), 109 (67).

4-Fluoro-2-methoxy-5-(2-propenyl)phenol (II) and 5-fluoro-2-methoxy-4-(2-propenyl)phenol (III) Fluorinated methyl eugenol I (980 mg, 5 mmol) was stirred with boron trichloride (6 ml of 1 M in CH_2Cl_2, 6 mmol) in dry CH_2Cl_2 (10 ml) at 0 to 25°C under N_2. After 24 hr, or when TLC showed no starting material present, the mixture was poured into ice-water and extracted with CH_2Cl_2. The combined extracts were washed with a saturated solution of sodium bicarbonate to pH ~7, then dried with sodium sulfate and concentrated. Flash chromatography with hexanes/ethyl acetate, 4:1 to 1:1, afforded a mixture of phenols II and III in a 57:43 ratio (592 mg, 65%). Phenols II and III were not separated by TLC but showed a baseline separation by GC on a DB-5 column, with phenol III being the first isomer to elute. The assignment of the isomeric phenol was done by ^{1}H NMR. GC-MS (m/z, %): II—182 (100, M^+), 167 (21), 155 (20), 151 (11), 149 (21), 139 (10), 121 (10), 109 (15), 91 (14). III—182 (100, M^+), 167 (32), 155 (18), 149 (15), 139 (8), 121 (11), 109 (15), 91 (11). ^{1}H NMR (500 MHz, $CDCl_3$): 3.28 (d, J=6.5, CH_2, II), 3.31 (d, J=6.5, CH_2, III), 3.82 (s, OCH_3, II), 3.83 (s, OCH_3, III), 5.04 (m, CH=), 5.36 and 5.67 (OH), 5.91 (m, CH_2=), 6.57 (d, $^3J_{HF}$=10.6, H-3, II), 6.61 (d, $^4J_{HF}$=6.9, H-3, III), 6.64 (d, $^3J_{HF}$=10.6, H-6, III), 6.71 (d, $^4J_{HF}$=6,9, H-6, II).

(E)-4-Fluoro-5-(3-hydroxy-1-propenyl)-2-methoxyphenol (IV) and (E)-5-fluoro-4-(3-hydroxy-1-propenyl)-2-methoxyphenol (V) Selenium dioxide (200 mg, 1.8 mmol) was dissolved in hot water (0.2 ml; ~80°C) and treated with a solution of phenols (II+III, 318 mg, 1.75 mmol) in dioxane (1.6 ml). The resulting mixture was heated in a water bath

(~85°C) for 15 min, then diluted with water (8 ml) and extracted with CH_2Cl_2 (4×5 ml). The combined organic extracts were washed with water (2×2 ml), brine (2×2 ml) and then dried with $MgSO_4$. The solution was concentrated, and the remainder was flash-chromatographed with hexanes/ethyl acetate, 3:4, to furnish some unreacted starting material (53 mg), then phenol IV (20 mg, R_f 0.44, hexanes/ethyl acetate, 1:2), a mixture of IV and V (16 mg) and, finally, phenol V (11 mg, R_f 0.38, hexanes/ethyl acetate, 1:2,). GC-MS (*m/z*, %): IV (Rt 14.27 min) – 198 (77, M^+), 155 (100), 142 (36), 140 (9), 137 (14), 127 (8), 121 (9), 109 (18); V (Rt 13.83 min) – 198 (71, M^+), 155 (100), 142 (36), 140 (8), 137 (12), 127 (10), 109 (16). ^{1}H NMR (500 MHz, $CDCl_3$ + CD_3OD, IV): 3.77 (s, OCH_3), 4.17 (dd, J=6.0, 1.0, CH_2), 6.15 (dt, J=16.0, 5.5, H-2′), 6.49 (d, $^3J_{HF}$=11.5, H-3), 6.57 (br. d, J=16.0, H-1'), 6.87 (d, $^4J_{HF}$=7.0, H-6). ^{1}H NMR (500 MHz, CD_3CN, V): 3.84 (s, OCH_3), 4.03 (dd, J=6.0, 1.5, CH_2), 6.25 (dt, J=16.0, 6.0, H-2′), 6.59 (d, $^3J_{HF}$=11.5, H-6), 6.63 (br. d, J=16.0, H-1'), 7.04 (d, $^4J_{HF}$=7.5, H-3).

Insects Laboratory reared oriental fruit flies (strain: Punador initiated in 1988–1989 and monitored by mating and survival tests) were obtained from the USDA-ARS-PBARC rearing facility in Honolulu, Hawaii. Pupae (60 ml) were placed in a screened aluminum cage (30×30×30 cm) and allowed to emerge. Flies were given water, sugar, and hydrolyzed yeast protein and held at 25–26°C, 50–70% relative humidity and a 12/12 hr L/D cycle before using in laboratory trials. Field bioassays were conducted with sterile laboratory reared oriental fruit fly, obtained as pupae, from a laboratory colony at the USDA-ARS-PBARC rearing facility in Honolulu, HI. Emerged adults were kept in an insectary at 24–27°C, 65–70% RH, and a photoperiod of 12:12 hr (L:D). Adults were fed water and a diet of three parts sucrose, one part protein yeast hydrolysate (Enzymatic, United States Biochemical Corporation, Cleveland, OH), and 0.5 part torula yeast (Lake States Division, Rhinelander Paper Co., Rhinelander, WI). Ten–eleven-day old, sexually mature, flies were used in the field studies.

Bioassays

Laboratory Feeding and Metabolism In Lab Trial 1, 50 laboratory-reared mature (9–10-day-old) oriental fruit fly males were placed in five ~1-l plastic containers (10/container) equipped with water inserts and screened covers. Flies were offered 10 μl of ME (2×5 μl) on a slip of filter paper (25×5 mm) taped to a blank water vial. Another 50 flies were offered the fluorine analog I (10 μl/cage), and the last group of 50 males were presented with a filter paper containing 10 μl water. Behavioral observations (landing and feeding on the source) were recorded every 5 min for 1 hr, then filter papers were removed, water containers were introduced, and the flies were left overnight with a sugar cube on the cover available for consumption. Numbers of dead flies was counted at 24 and 48 hr. Eighteen hours post feeding, the rectal glands of ten flies each fed with ME and I (cages where the landings occurred most), as well as ten control flies were dissected and placed in conical plastic vials with 20 μl ethanol. The glands were crushed with a wire rod, sealed with parafilm and centrifuged at ~4,000 rpm for 10 min. The supernatants were diluted in the same vials with ethanol to 100 μl and analyzed by GC-MS in splitless mode by injection of 1 μl solutions. External standards of CA, DMP, and V were used for quantification. The remainders of the dissected flies were individually placed in 20 μl ethanol, crashed, then flash-chromatographed on a short SiO_2 column (30×5 mm) with hexanes/ethylacetate, 3:1. The fractions containing unmetabolized ME and fluorine analog I were further combined, concentrated to 1 ml, and quantified by GC using external standards.

Lab Trial 2 was conducted analogously to Lab Trial 1 with the exception of the treatments presented which were 1 μl of ME (10 μl of 10% *v/v* hexane solution), 1 μl of fluorine analog I, and water as control. In Lab Trial 3, the doses of treatments were reduced to 0.1 μl. No dissections and metabolism studies were conducted in Lab Trials 2, and 3.

Field Attractiveness and Release Rate

Dispenser Preparation and Trapping For each test, cotton dental wicks (9 mm diam × 14 mm long) were treated with aliquots of a hexane-acetone stock solution (2:1 *v/v*) containing a test compound. Three chemicals, fluorine analogs I, ME, and FME, were tested in Field Trial 1 while four chemicals, I, ME, FME, and DMP were tested in Field Trial 2. Each wick was treated with 250 μl of the stock solution, a volume calculated to deliver ca. 10 mg of a test chemical. After allowing the solvent to evaporate (20–25 min), wicks were enclosed individually in screen-covered plastic baskets (to prevent feeding consumption by oriental fruit fly males). By using metal clips, baskets that contained wicks were hung inside Jackson traps. Each Jackson trap had a sticky basal insert and two 2.5-cm dog collar strips (Bansect® flea and tick collar for dogs, ®ConAgra Pet Products, Richmond, VA, USA) containing a knock down toxicant (15% Naled by weight; ~0.38 g/strip). Bioassays with releases of 10–11-day old laboratory-reared oriental fruit fly adults were conducted in a macadamia (*Macadamia integrifolia* Maiden and Betche) orchard in the vicinity of Hilo, Hawaii. A low-density population of wild oriental fruit flies subsisting on wild hosts inside and outside this orchard occurred naturally. The experimental design was randomized complete block with six (Field Trial 1) or five

(Field Trial 2) replications. The interblock distance was 10 m, with treatments in each block placed 8 m apart. Approximately 6,000 sterile oriental fruit fly males were uniformly released per block at the start (5 Sept., 1995 for Field Trial 1; 1 Nov., 1995 for Field Trial 2) of each of two successive 24-hr periods. After 24 hr, inserts with trapped adults from each trap were recovered, and new inserts were placed in each trap. All traps were gathered and moved to a new trapping grid of exactly the same configuration at least 500 m away (moving the traps to another trapping grid was done to approximate similar, uniform density of adults in the trapping grid for 0–24 and 24–48 hr). After 48 hr, wicks and inserts were recovered from each trap and immediately brought back to the laboratory. The total number of trapped oriental fruit fly males was determined for each retrieved insert.

Weathering of Wicks and Residue Analyses At the time of the initial wick treatment for Field Trial 2, five treated wicks of each treatment (time 0 wicks) were placed in individual glass vials, sealed with Teflon tape, and frozen. Concurrent with the distribution of treatments in Field Trial 2 (described above), the same number of additional traps of each treatment were emplaced in a randomized complete block wick weathering grid. Wicks recovered from these grids after 24 hr, together with time 0 wicks and wicks collected after 48 hr from the trap catch grid, were used to estimate the amount of compound that had volatilized from the wicks during 0–24 hr and 24–48 hr of Field Trial 2. As with wicks used in the bioassays, weathering wicks were enclosed in screen-covered baskets that were hung inside Jackson traps. Following retrieval at 24 and 48 hr, wicks were placed individually in 25-ml glass vials, sealed with Teflon tape, and shipped to Beltsville, MD, USA, for residual analyses. Gas chromatography-quality acetone was added to each vial (15 ml), vials were stoppered, and wicks were allowed to steep overnight at 25°C. A predetermined amount of an internal standard, 1,2-dimethoxy-4-ethylbenzene (synthesized following Khrimian et al. 1993) was added to each vial, and the extracts were analyzed by gas chromatography.

Data Analysis Mean data from the metabolism study (Lab Trial 1) were analyzed using PROC GLM followed by a Tukey's HSD test for mean separation. Significant differences were determined at the $P<0.05$ level. Analysis was run on SAS version 8.2 (SAS Institute 1990). Mean data from the feeding study were analyzed with a two-way ANOVA. These analyses were conducted by using STATISTICA (Statsoft 2001). Trap catch results from the field bioassays were square root transformed ($\sqrt{[x+0.5]}$; Sokal and Rohlf 1981) and then subjected to ANOVA with Tukey's HSD used for means separation (SAS Institute 2002). Treatment volatilization was analyzed by ANOVA for the 0–24-hr and 24–48-hr periods, with Tukey's HSD used for means separation (SAS Institute 2002).

Results

Syntheses of Ring-fluorinated Analog I and Metabolite V We straightforwardly synthesized the targeted fluorinated benzene I (Scheme 1, panel A) from 1-chloromethyl-2-fluoro-4,5-dimethoxybenzene by using a coupling proce-

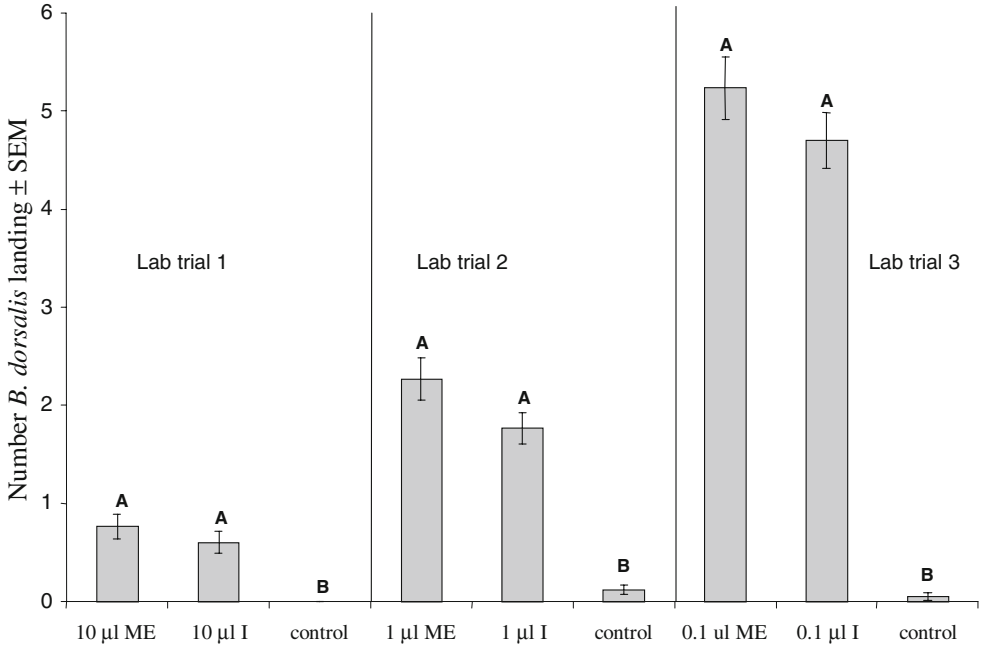


Fig. 1 Landing and feeding of *B. dorsalis* males in a cage experiments with ME and fluorine analog I. Ten males were offered two concentrations of both attractants in five replicates. A two-way ANOVA showed a significant effect of dose (df=2.24; F=76.6, p<0.001) but no differences between treatments within trials, ME and I (df=1.24; F=1.91; p=0.18). Treatment dose was nonsignificant (df=2.24; F=0.16, p=0.85). *Means followed by the same letter* are not significantly different. (Statsoft Inc, 2001)

dure developed earlier for alkyl-substituted analogs of methyl eugenol (Khrimian et al. 1993). Fluorinated benzene I was converted to V with a two-step procedure depicted in Scheme 1, panel A. First, compound I was demethylated with boron trichloride in methylene chloride (Dean et al. 1966) to give a 57:43 mixture of two phenols, baseline separated by GC on a DB-5 column but not by TLC. Thus, the prospect of isolating the individual phenols was uncertain, and their structural assignment presented a certain challenge. Fortuitously, signals from the aromatic protons of the two isomeric phenols were clearly separated in the ^{1}H NMR spectrum of the mixture recorded at 500 MHz, which allowed us to apply a nuclear Overhauser effect (NOE) for their assignments. A NOE difference spectrum obtained by irradiating CH_3O resonance (at about the same frequency for both isomeric phenols) showed an enhancement of two signals at δ 6.57 (d, J=10.6 Hz) and 6.61 (d, J=6.9 Hz). Based on the literature evidence of larger proton-fluorine coupling constant in the *ortho* position ($^3J_{HF}$) compared to that of in the *meta* position ($^4J_{HF}$), and the actual values of these interactions (Silverstein et al. 1980), we assigned the first doublet (of higher intensity) to the proton on C-3 of phenol II and the second to the proton on C-3 of phenol III. Conversely, the signals at δ 6.64 (d, J=10.6 Hz) and 6.71 (d, J=6.9 Hz) were assigned to protons on C-6 of phenols III and II, respectively. Thus, phenol III, needed for the completion of the synthesis of the metabolite V, was a minor component in the mixture. In the last step, phenols II and III were oxidized with selenium dioxide in an aqueous dioxane (Pan et al. 1980) to give a mixture of diols, from which pure IV and V were isolated by flash chromatography. The NOE difference technique described above was used again for structural assignments. In the case of V, irradiation of CH_3O led to the enhancement of the doublet at δ 7.04 ($^4J_{HF}$=7.5 Hz) from the proton on C-3, positioned *meta* to the fluorine atom, whereas in diol IV a NOE effect from CH_3O resonance occurred on the proton in the *ortho* position to fluorine (δ 6.49 d, $^3J_{HF}$=11.5 Hz). *Trans*-configuration of the double bonds in both IV and V was evident from $^3J_{HH}$=16.0 Hz and concurrent with the chemistry of SeO_2 oxidation of 3-phenylpropenes (Pan et al. 1980). Synthetic diol V, based on a GC retention time and mass-spectrum, matched the compound found in rectal glands of oriental fruit fly males fed with I. It was also used as a standard for quantitative analysis of gland extracts.

Feeding and Metabolism In all three experiments (Fig. 1) conducted with different doses, there was no statistically significant difference between fluorine analog I and ME in

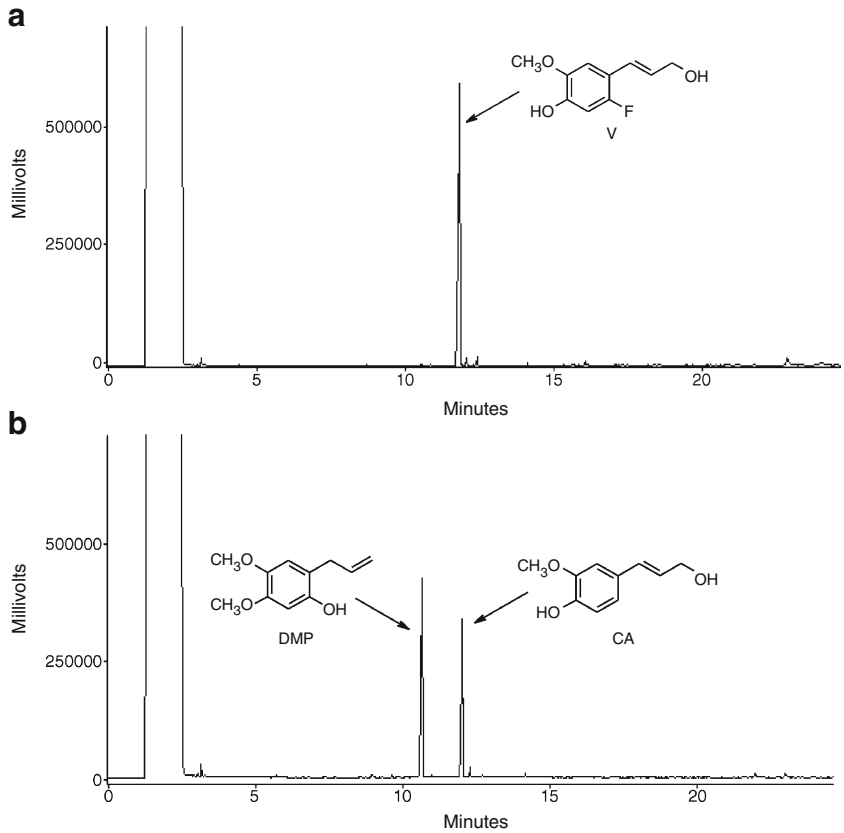


Fig. 2 Gas chromatograms (*HP-5, FID*) of gland extracts of individual oriental fruit fly males fed with: **A** fluorine analog I (Table 1, species # 3), and **B** methyl eugenol (Table 1, species # 1)

Table 1 Amounts of metabolites and unmetabolized ring-fluorinated analog I and ME in oriental fruit fly males from Lab Trial 1

Fluorine analog I				ME				
Insect no.	Metabolite V, μg[a]	Unmetabolized I, μg[b]	V, molar %	Metabolites, μg		Unmetabolized ME, μg[b]	CA, molar %	Total metabolites, molar %
				CA[a]	DMP[a]			
1	64.9	141.5	45.4	16.1	17.3	55.3	18.3	36.5
2	28.2	26.4	51.4	16.3	19.6	302.7	4.8	10.1
3	121.2	135.0	47.1	21.8	14.8	2.1	57.9	94.4
4	52.4	75.1	40.8	24.0	24.6	285	7.2	14.0
5	74.2	26.6	73.4	43.1	20.3	276.5	12.6	18.1
6	99.6	48.4	67.1	4.2	4.2	162.9	2.4	4.7
7	26.6	1.5	94.6	10.6	9.0	66.6	12.3	22.0
8	31.9	1.5	95.4	7.3	4.8	53.7	11.1	17.8
9	72.5	34.0	67.9	16.0	14.6	112.6	11.2	20.6
10	78.3	68.3	53.2	16.8	8.2	54.2	21.2	30.8
Total	650	558		176	137	1372		
Mean ± SEM	65.0±9.9	55.8±15.7	63.6±6.2a[c]	17.6±3.4	13.7±2.2	137.2±35.5	15.9±5.0b[c]	26.9±8.1b[c]

[a] GC analysis of glands
[b] GC analysis of other body parts
[c] Means followed by different letters are significantly different at the 0.05 level, PROC GLM, Tukeys test (SAS Institute 1990)

male *B. dorsalis* landing and feeding on the source (F=1.91; P=0.18). However, there was a difference between doses (df=2.24; F=76.6, P<0.001), with the most landings recorded in Lab Trial 3 (0.1 μl, ~5 landings in average out of 10). The least number of landings were observed in Lab Trial 1 (10 μl, <1 landing), and Lab Trial 2 (1.0 μl) was in the intermediate position (~2 landings). No fly landings were observed in the control cage in Lab Trial 1, and landings in control cages in Lab Trial 2 and 3 were less than in cages containing I and ME (F=76.6; P<0.001). Flies fed with compound I did not suffer significantly higher levels of mortality than those fed with ME. No mortalities were observed after 24 and 48 hr in low-dose Lab Trials 2 and 3 with either treatment. In Lab Trial 1, two flies fed with compound I were dead after 24 hr and total of five flies were dead after 48 hr. In the same high-dose experiment, the numbers of dead flies fed with ME after 24 and 48 hr were five and eight, respectively.

Results of chemical analyses of rectal glands and remaining body parts of ten flies from Lab Trial 1 are presented (Fig. 2, Table 1). A representative gas-chromatogram of a gland extract of an individual *B. dorsalis* male fed with I (Fig. 2, panel A) reveals essentially a single compound that matches the synthetic diol V by retention time and mass-spectrum. As expected, the flies fed with ME produced the well-described metabolites, coniferyl alcohol and 4,5-dimethoxy-2-(2-propenyl)phenol (Fig. 2, panel B). Unmetabolized test chemicals were not found in rectal glands but were present in the extracts of fly remains after dissection. No phenylpropanoids were found in the control flies. As shown in Table 1, total intake of test materials by ten flies offered 10 μl of each I or ME were similar: 1208 (650 +558) μg for compound I and 1509 (137+1372) μg for ME. However, a remarkable difference between the treatments was observed in the amounts of metabolites relative to intakes. At this feeding level, *B. dorsalis* males were able to metabolize, after 18 hr, on average ~64% of the fluorine analog I and only ~27% of ME (~16% of the ME intake was converted to CA). It appears, however, that at a lower consumption level (20–40 μg) flies can almost completely metabolize both compounds (Table 1, rows 7, 8 for I, and row 3 for ME).

Field Bioassay Trap capture of males from field bioassays are presented in Fig. 3 (Field Trial 1) and Fig. 4 (Field Trial 2). There were differences in trap captures by treatment in Field Trial 1 at both 24 hr (F=23.7, df=2, 15, P<0.001) and at 48 hr (F=22.2, df=2, 15, P<0.001); and in Field Trial 2 at both 24 hr (F=14.5, df=3, 16, P<0.001) and at 48 hr (F=41.5, df=3, 16, P<0.001). In Field Trial 1, at both 24 and 48 hr, there was no significant difference in captures between traps baited with ME and traps baited with FME. However, catches at traps baited with I were statistically less than at traps baited with either ME or FME. In Field Trial 2, at both 24 and 48 hr, there was again no significant difference in catches between traps baited with ME or with FME. Captures at traps baited with I were significantly less than at traps baited with methyl eugenol (but not FME) at 24 hr and were significantly less than at traps baited with either ME or FME at 48 hr. Catches at traps baited with

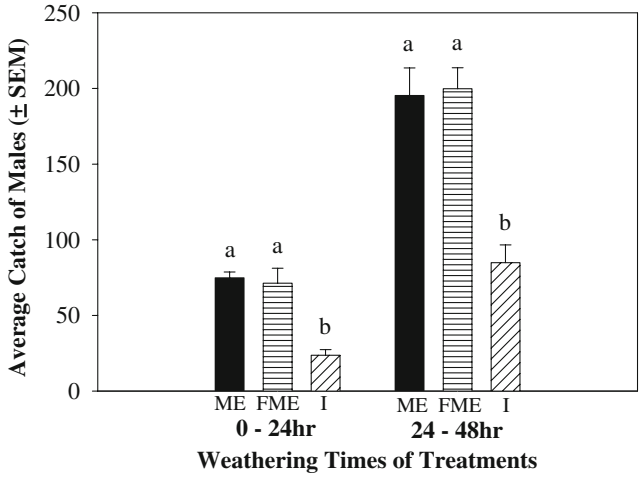


Fig. 3 Field Bioassay 1, September 1995. Average catch of male oriental fruit fly adults 0–24 and 24–48 h after field deployment of Jackson traps baited with methyl eugenol (*ME*), ring-fluorinated analog (*I*), and side-chain fluorinated analog (*FME*). *Columns within the same time period having the same letter* are not significantly different at the α=0.05 level

DMP were significantly less than catches with all other treatments at both 24 and 48 hr. There were differences in volatilization by treatment in both the 0–24 hr (F=289.4, df=3, 16, P<0.001) and 24–48 hr (F=116.8, df=3, 16, P<0.001) time periods. Release rates of I and ME from cotton wicks were not significantly different at either 24 or 48 hr, whereas release of FME was significantly lower than those of I and ME at both 24 and 48 hr. Finally, the release of DMP was significantly lower than those of any other treatments at both time intervals.

Discussion

Our laboratory studies demonstrated that the ring-fluorinated analog I is perceived by *B. dorsalis* males as an analog of natural phenylpropanoid methyl eugenol. In close range experiments, flies were attracted to and fed on analog I and methyl eugenol at similar rates. At the highest concentration of test materials in cage experiments, flies rarely touched the source. This behavior is similar to insect avoidance, observed under field conditions, of traps baited with a large amount of a pheromone (Zhang and Amalin 2005). Although few flies directly contacted the filter papers, they still absorbed appreciable amounts of vaporized I and ME. Consumption of fluorine analog I did not result in an increased mortality in *B. dorsalis* males compared to flies fed with ME, which has been reported to cause death in the oriental fruit fly when ingested in large amounts (Steiner 1952). Thus, the fitness of *B. dorsalis* males evidently is linked (among other factors) to their ability to efficiently metabolize phenylpropanoids.

Introduction of the fluorine atom on the aromatic ring of ME at a position which undergoes enzymatic hydroxylation (Scheme 1, panel B) resulted in a complete blocking of this biotransformation pathway. While this was not particularly surprising, the observation that blocking one pathway did not result in an overall slowing of metabolism was interesting. Oriental fruit fly males demonstrated a remarkable ability to shift metabolism of the consumed fluorine analog I through a side-chain hydroxylation pathway (Smith et al. 2002), leading to the fluorinated (*E*)-coniferyl alcohol V. In fact, the rate of metabolism of I was about twice that of ME under our experimental conditions (Table 1, last columns). Our previous studies with FME (Scheme 1, panel B) showed that the fluorine introduction slowed overall metabolism and specifically impeded formation of CA (Khrimian et al. 2006). Thus, the incorporation of the fluorine atom at the aromatic ring and at the

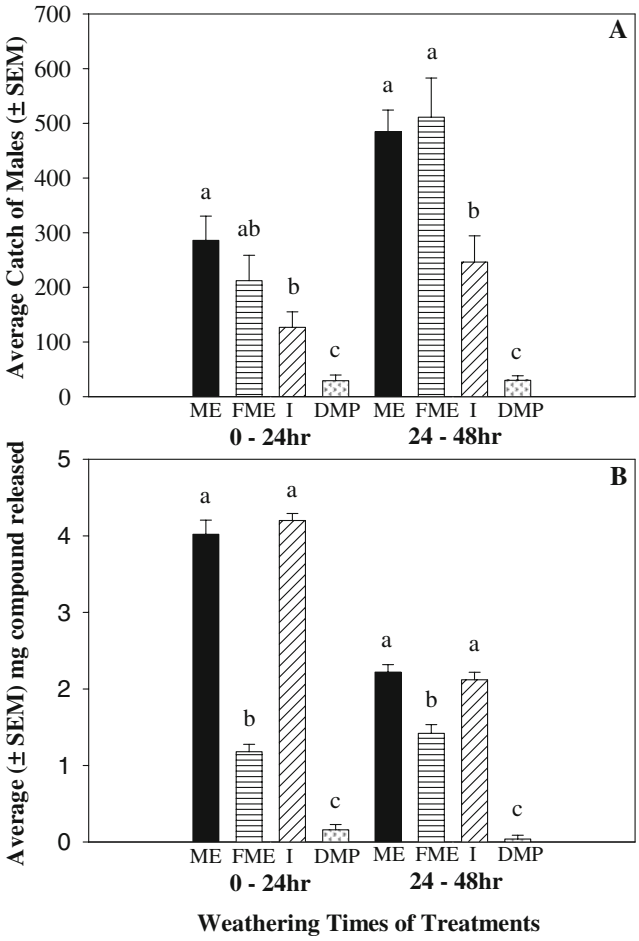


Fig. 4 Field Bioassay 2, November 1995. Average catch of male oriental fruit fly adults (**A**) and release of test chemicals (**B**) 0–24 and 24–48 h after field deployment of Jackson traps baited with methyl eugenol (*ME*), ring-fluorinated analog (*I*), side-chain fluorinated analog (*FME*), and dimethoxyphenol (*DMP*). *Columns within the same time period having the same letter* are not significantly different at the α=0.05 level

terminal carbon atom of the double bond of ME had opposite effects on the side-chain metabolic hydroxylation.

Although *B. dorsalis* males were attracted strongly to and fed on fluorine analog I in a cage experiment, field attractiveness of male oriental fruit fly to I was markedly lower than to ME. In both field bioassays, traps baited with compound I captured less than 50% of flies caught in traps baited with ME. Release rates of fluorine analog I and ME were similar (Fig. 4), so trap catches provide a valid comparison of field attractiveness of these two compounds. In contrast, the evaporation of DMP from a cotton wick was substantially (~30 times) lower compared to I, making a linkage of trap catches to attractiveness tenuous. Even at a much slower release rate, however, the average number of males caught in traps baited with DMP was only 4–8 times lower than that in analog I-baited traps. Thus, a fluorine substitution for the hydroxy group at the aromatic ring of DMP increased the volatility but did not improve attractiveness to *B. dorsalis*. Metcalf et al. (1975, 1981) studied molecular parameters of attractants and olfaction in *B. dorsalis* that involved a number of substituted *ortho*-dimethoxybenzenes. Several postulates were derived from those studies: (a) unshared electron pairs of the *ortho*-dimethoxy groups were essential for receptor depolarization, yet the methoxy group in *para* position to allyl group of ME seemed to be of a primary importance; (b) electron-donating side-chain groups produced stronger attractants than those withdrawing electrons.

We did not find in the literature any activity correlations with the substitution pattern of DMP and fluorine analog I, but because the fluorine atom is isosteric with hydrogen (and being such has been widely pursued for altering the properties of biologically active substances), it seems that it is the electronic factor of fluorine substitution that is responsible for a reduced attractiveness of analog I compared to ME. A strong electronegative effect of the fluorine atom should decrease electron density about the CH_3O groups in analog I but would primarily affect the methoxy group in the *para*-position to F (*meta* to allyl group), thus diminishing but not abolishing the attraction to *B. dorsalis*. In any case, a moderate field attractiveness combined with the propensity to side-chain metabolic oxidation (linked to a carcinogenicity) makes the analog I an unlikely replacement for ME as an attractant for the oriental fruit fly. We, however, were pleased with the performance of FME in two short field bioassays, which confirmed our earlier findings (Khrimian et al. 1994; Liquido et al. 1998) that this compound is not only equally attractive to *B. dorsalis* but also has added value as a more persistent lure.

In summary, we developed a ring-fluorinated analog of methyl eugenol that is highly attractive to the oriental fruit fly males in a laboratory cage experiment but is only moderately active in the field. This new fluorine analog is metabolized faster than methyl eugenol after being consumed by *B. dorsalis* males and produces almost exclusively fluorinated (*E*)-coniferyl alcohol.

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