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Phenylpyrazole Insecticide Photochemistry, Metabolism, and GABAergic Action: Ethiprole Compared with Fipronil

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Ethiprole differs from fipronil, the major phenylpyrazole insecticide, only in an ethylsulfinyl substituent replacing the trifluoromethylsulfinyl moiety. This study compares their photochemistry, metabolism, action at the γ-aminobutyric acid (GABA) receptor, and insecticidal potency. On exposure to sunlight as a thin film, ethiprole undergoes oxidation (major), reduction, and desethylsulfinylation but not desulfinylation whereas the major photoreaction for fipronil is desulfinylation. Metabolic sulfone formation is more rapid with ethiprole than fipronil in human expressed CYP3A4 in vitro and mouse brain and liver in vivo. High biological activity is observed for the sulfide, sulfoxide, sulfone, and desulfinyl derivatives in both the ethiprole and the fipronil series in GABA receptor assays (human recombinant β3 homomer and house fly head membranes) with [^{3}H]EBOB and in topical toxicity to house flies with and without the P450-inhibiting synergist piperonyl butoxide. On an overall basis, the ethiprole series is very similar in potency to the fipronil series.

KEYWORDS: Ethiprole; fipronil; GABA receptor; metabolism; phenylpyrazole insecticide; photochemistry

INTRODUCTION

Ethiprole, 5-amino-3-cyano-1-(2,6-dichloro-4-trifluoromethylphenyl)-4-ethylsulfinylpyrazole, is a new 1-phenylpyrazole insecticide (*1*) effective against a broad spectrum of chewing and sucking insects with pronounced plant systemic activity and potential uses on rice, cotton, corn, alfalfa, peanuts, and soybeans (*2*) as well as stored grain insect pests (*3*). Fipronil, the 4-trifluoromethylsulfinyl analogue (*4*), is one of the most important insecticides for control of soil insects on corn (*5*) and fleas on cats and dogs (*6*). The change in the single substituent, i.e., ethylsulfinyl vs trifluoromethylsulfinyl (**Figure 1**), alters the lipophilicity and electronic properties and potentially also the photochemical and metabolic fate, effectiveness, and toxicology. Fipronil is converted to sulfone, sulfide, desulfinyl, and destrifluoromethylsulfinyl derivatives and fipronil amide as photoproducts and to the sulfone metabolite (*7*−*11*) thereby serving as a model for ethiprole.

The toxicity of phenylpyrazoles to insects and mammals is attributable to their action at the GABA receptor as noncompetitive blockers of the GABA-gated chloride channel (*12*−*15*). This target site is conveniently examined with [^{3}H]EBOB in binding assays with house fly (*Musca domestica*) head membranes (*12*, *16*) and human recombinant β3 homomeric receptor (*17*, *18*). House flies are more sensitive than mice to fipronil derivatives in comparative studies (*8*, *12*). The potency of phenylpyrazoles and other GABA-gated chloride channel blockers in the [^{3}H]EBOB assay generally correlates with toxicity to house flies (*12*, *16*) and also with selectivity if the native mammalian receptor is used (*17*, *18*).

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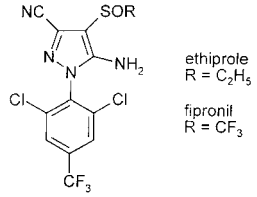

Figure 1. Structures of ethiprole (R═C_2H_5) and fipronil (R═CF_3).

This study compares the chemistry and action of ethiprole and fipronil and their photoproducts and metabolites (**Figure 2**) relative to effectiveness and safety. Compound designations are used for ease of comparison in the ethiprole (E) and fipronil (F) series by coupling with the 4-substituent of the pyrazole, e.g., ethiprole is E−SOC_2H_5 and desulfinyl fipronil is F−CF_3 (**Figure 2**).

MATERIALS AND METHODS

Chemicals. Sources for the chemicals were ethiprole (>99% purity) from Aventis Crop Science SA (CRDL-Lyon, France); α-endosulfan and fipronil (>98% purity) from Chem Service Inc. (West Chester, PA), fipronil amide (F−amide) prepared by hydrolysis of fipronil as described later for ethiprole amide (E−amide) from ethiprole, [^{3}H]-EBOB (30 Ci/mmol, >97% radiochemical purity) from NEN Life Science Products (Boston, MA), piperonyl butoxide (PB) from Aldrich Chemical Co. (Milwaukee, WI), ethyl 1,3-dicyanopropionate prepared from ethyl cyanoacetate, paraformaldehyde, and potassium cyanide in ethanol (*19*).

Analyses. For gas chromatography/mass spectrometry (GC/MS), a Hewlett-Packard 5890 series II gas chromatograph was used with a model 5971 mass selective detector fitted with a 15 m × 0.25 mm i.d. × 0.25 μm film DB5 column (J&W Scientific, Folsom, CA) and operated in the electron impact and full scan mode (50−550 *m*/*z*). The injector and detector were at 200 and 280 °C, respectively. The sample

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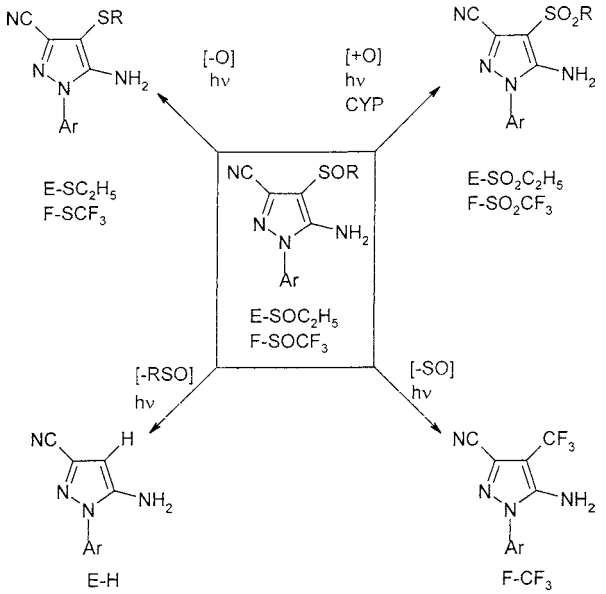
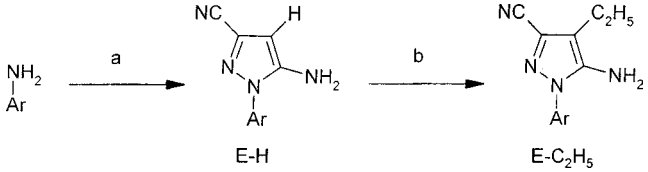


Figure 2. Photochemical and metabolic modifications of the pyrazole substituents of ethiprole (E–SOC_2H_5) and fipronil (F–$SOCF_3$); R = C_2H_5 or CF_3; Ar = 2,6-dichloro-4-trifluoromethylphenyl. The major photocleavage reaction of ethiprole is desethylsulfinylation and of fipronil is desulfinylation. CYP refers to products in CYP3A4 systems in vitro or in mouse brain in vivo. Ethiprole amide and fipronil amide are not observed as photoproducts or metabolites in this study.

Scheme 1 [a]



[a] Ar = 2,6-dichloro-4-trifluoromethylphenyl. (a) (1) $NaNO_2$, H_2SO_4; (2) $ArNH_2$, CH_3CO_2H; (3) ethyl 2,3-dicyanopropionate, CH_3CO_2H, H_2O; (4) NH_4OH, CH_2Cl_2. (b) (1) *N*-Iodosuccinimide, CH_3CN; (2) $Bu_3SnCH{=}CH_2$, $Pd(PPh_3)_4$, DMF; (3) H_2, Pd/C, EtOAc.

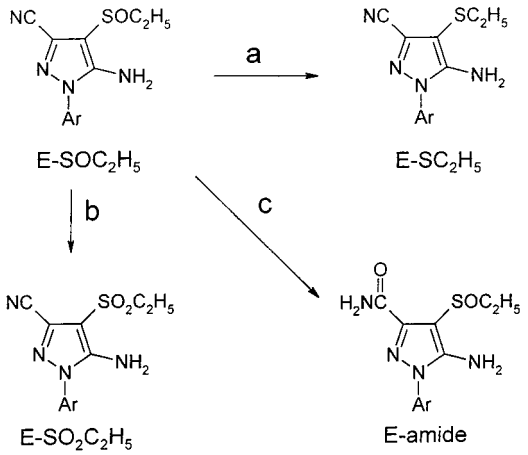
(1 μL) was injected in the splitless mode, and the oven temperature was programmed as follows: 60 °C for 1 min, raised to 250 °C (10 °C/min), and held 3 min. Helium was the carrier gas at 54.4 KPa. A DB1 column with a higher temperature was used for the fipronil series as detailed later. Quantitation of the photoproducts and metabolites was based on standard curves for the authentic compounds as total ion current.

For HPLC, a Hewlett-Packard model 1050 liquid chromatograph was used fitted with a diode array detector model HP1040M and a 250 mm × 4.6 mm × 5 μm Beckmann Ultrasphere ODS column. Isocratic development was with water−acetonitrile (1:1). The sample injection volume was 100 μL with a flow rate of 1 mL/min. Quantitative analyses involved peak area comparisons with synthetic standards and absorbance measurements at 280 nm.

Preparation of Ethiprole Derivatives. The synthesis routes are given in **Scheme 1** for E−H and E−C_2H_5 and **Scheme 2** for E−SC_2H_5, E−$SO_2C_2H_5$, and E−amide. The GC/MS and high-performance liquid chromatography (HPLC) characteristics are reported in **Table 1**.

Desethylsulfinyl Ethiprole (E−H) [5-Amino-3-cyano-1-(2,6-dichloro-4-trifluoromethylphenyl)pyrazole]. E−H was prepared by a reported method (*20*) with slight modification. Sodium nitrite (0.38 g, 5.5 mmol) was placed in a 10 mL round bottom flask and stirred magnetically. Concentrated sulfuric acid (1.4 mL) was added dropwise and mixed for 15 min before acetic acid (1.2 mL) was added. After the reaction had cooled to 20 °C, 2,6-dichloro-4-trifluoromethylaniline (1.06 g, 4.6 mmol) dissolved in acetic acid (5 mL) was added at 1 mL/min. The mixture was then heated in an oil bath at 50 °C for 30 min and poured

Scheme 2 [a]



[a] Ar = 2,6-dichloro-4-trifluoromethylphenyl. (a) NaI, acetone, trifluoroacetic anhydride. (b) $KMnO_4$, acetone. (c) NaOH, C_2H_5OH, H_2O.

into a solution of ethyl 2,3-dicyanopropionate (0.70 g, 4.6 mmol) in acetic acid (3 mL) and water (6 mL) at 10 °C. After the mixture was stirred for 1 h at room temperature, water and dichloromethane were added and the aqueous layer was extracted twice with dichloromethane. These combined organic layers were vigorously stirred with ammonium hydroxide (30%, 30 mL) overnight. The organic layer was washed with water and 1 N hydrochloric acid, dried with sodium sulfate, filtered, and concentrated to give E−H as a crude oil that was crystallized with dichloromethane/hexane (0.96 g, 66%); mp 141−142 °C (reported 140−142 °C).

Desulfinyl Ethiprole (E−C_2H_5) [5-Amino-3-cyano-1-(2,6-dichloro-4-trifluoromethylphenyl)-4-ethylpyrazole]. E−H was iodinated with *N*-iodosuccinimide in acetonitrile to give the corresponding 4-iodopyrazole, which underwent Stille coupling (*21*) [tri-*n*-butyl(vinyl)tin and tetratris(triphenylphosphine)palladium in dimethylformamide] to provide 5-amino-3-cyano-1-(2,6-dichloro-4-trifluoromethylphenyl)-4-ethenylpyrazole as previously described (*22*). Hydrogenation of this vinyl intermediate (14 mg) with 10% Pd/C (14 mg) in ethyl acetate (2 mL) at room temperature for 1 h gave after filtration and concentration the desired E−C_2H_5 (13 mg, 93%) as a white solid; mp 147−149 °C. ^{1}H NMR ($CDCl_3$): δ 7.78 (s, 2× aryl-H), 3.51 (s, NH_2), 2.56 (q, J = 7.6 Hz, CH_2), 1.28 (t, J = 7.6 Hz, CH_3). ^{13}C NMR ($CDCl_3$): δ 143.3, 136.5, 134.3 (q, J = 34 Hz), 127.4, 126.1, 122.0 (q, J = 270 Hz), 113.5, 111.2, 99.5, 16.3, 14.0.

Ethiprole Sulfide (E−SC_2H_5) [5-Amino-3-cyano-1-(2,6-dichloro-4-trifluoromethylphenyl)-4-ethylsulfenylpyrazole]. Following the procedure for reduction of fipronil to its sulfide (*23*), E−SOC_2H_5 (52 mg, 0.13 mmol) and sodium iodide (59 mg, 0.39 mmol) were dissolved in acetone (600 μL). Trifluoroacetic anhydride (55 μL, 0.39 mmol) was added dropwise to the stirred solution at 0 °C. The reaction was kept at 0 °C for 5 h, warmed to room temperature over 3 h, quenched with aqueous sodium thiosulfate, and extracted with ethyl acetate three times. The combined organic extracts were washed with water and brine. The crude residue obtained after drying with sodium sulfate and concentration was purified by preparative thin-layer chromatography (TLC) on silica gel with ethyl acetate:hexane (2:3) to give E−SC_2H_5 (32 mg, 64%); mp 156−158 °C. ^{1}H NMR ($CDCl_3$): δ 7.80 (s, 2× aryl-H), 4.20 (s, NH_2), 2.71 (q, J = 7.4 Hz, CH_2), 1.27 (t, J = 7.4 Hz, CH_3). ^{13}C NMR ($CDCl_3$): δ 149.8, 136.4, 135.5, 134.6 (q, J = 34 Hz), 132.6, 126.8, 121.9 (q, J = 270 Hz), 112.6, 96.3, 30.5, 15.0.

Ethiprole Sulfone (E−$SO_2C_2H_5$) [5-Amino-3-cyano-1-[2,6-dichloro-4-(trifluoromethylphenyl)-4-ethylsulfonylpyrazole]. E−SOC_2H_5 was oxidized with potassium permanganate in aqueous acetone to afford the corresponding sulfone analogous to our previous report for fipronil (*7*). E−SOC_2H_5 (150 mg, 0.38 mmol) in acetone (7 mL) was combined with magnesium sulfate (130 mg) in water/acetone (30 mL, 1:1). A solution of potassium permanganate (100 mg, 0.6 mmol) in water/acetone (20 mL, 1:1) was added to the vigorously stirred solution. After the mixture was stirred overnight at room temperature, concentrated

Table 1. GC/MS and HPLC Characteristics of Ethiprole and Its Derivatives

compd (exact mass)[a]	EI-MS, *m/z* (amu) (% relative abundance)	t_R (min) GC/MS[b]	t_R (min) HPLC[c]
E–H (321)	320 $[M]^+$ (100), 285 $[M - Cl]^+$ (25), 213 $[C_7H_2F_3Cl_2]^+$ (23), 250 $[M - Cl_2]^+$ (22), 258 $[M - (CN)Cl]^+$ (20), 179 $[C_7H_3F_3Cl]^+$ (19), other ions 240 (19), 233 (18)	14.78	11.15
E–C_2H_5 (349)	333 $[M - CH_3]^+$ (100), 348 $[M]^+$ (33), 213 $[C_7H_2F_3Cl_2]^+$ (15), 179 $[C_7H_3F_3Cl]^+$ (14), other ions 246 (28), 53(15), 157 (14)	15.18	15.39
E–SC_2H_5 (381)	255 $[C_6H_2(Cl_2)(CF_3)(N_2CH_2)]^+$ (100), 352 $[M - C_2H_5]^+$ (95), 380 $[M]^+$ (72), 213 $[C_7H_2F_3Cl_2]^+$ (58), 179 $[C_7H_3F_3Cl]^+$ (26), 77 $[C_6H_5]^+$ (20), 317 $[M - C_2H_5(Cl)]^+$ (16), other ions 290 (24), 143 (15)	16.99	22.35
E–SOC_2H_5 (397)	367 $[M - C_2H_5]^+$ (100), 213 $[C_7H_2F_3Cl_2]^+$ (49), 255 $[C_6H_2(Cl_2)(CF_3)(N_2CH_2)]^+$ (28), 352 $[M - OC_2H_5]^+$ (18), 77 $[C_6H_5]^+$ (18), 179 $[C_7H_3F_3Cl]^+$ (16), 396 $[M]^+$ (15), 320 $[M - SOC_2H_5]^+$ (13)	17.52	10.03
E–$SO_2C_2H_5$ (413)	320 $[M - SOC_2H_5]^+$ (100), 213 $[C_7H_2F_3Cl_2]^+$ (60), 255 $[C_6H_2(Cl_2)(CF_3)(N_2CH_2)]^+$ (28), 77 $[C_6H_5]^+$ (28), 412 $[M]^+$ (20), 179 $[C_7H_3F_3Cl]^+$ (17), other ions 241 (30), 324 (18), 143 (15)	18.64	13.63
E–amide (415)	385 $[M - C_2H_5]^+$ (100), 255 $[C_6H_2(Cl_2)(CF_3)(N_2CH_2)^+$ (27), 213 $[C_7H_2F_3Cl_2]^+$ (16), 368 $[M - C_2H_6O]^+$ (18), 414 $[M]^+$ (15)	19.48	5.61

[a] Cl = 35. [b] GC/MS conditions for the ethiprole series: DB5, 15 m column, 60 °C for 1 min and then 10 °C/min to 250 °C and held for 3 min. GC/MS conditions for the fipronil series: DB1, 30 m column, 150 °C for 1 min and then 10 °C/min to 250 °C and held for 14 min; t_R values 9.67, 8.21, 9.57, 9.77, 10.82, and 12.44 min for F–H, F–CF_3, F–SCF_3, F–$SOCF_3$, F–SO_2CF_3, and F–amide, respectively. [c] HPLC conditions: ODS column with water–acetonitrile (1:1) at 1 mL/min. In the fipronil series, for comparison, t_R values are 18.5 min for F–$SOCF_3$ and 28.8 min for F–SO_2CF_3.

hydrochloric acid was added dropwise to destroy excess oxidant. This solution was extracted with diethyl ether three times, and the combined ether extracts were dried over sodium sulfate. The solvent was evaporated, and the white solid obtained was recrystallized from ethyl acetate/hexane to give E−$SO_2C_2H_5$ (145 mg, 93%); mp 183−185 °C. ^{1}H NMR ($CDCl_3$): δ 7.84 (s, 2× aryl-H), 5.16 (s, NH_2), 3.3 (q, J = 7.4 Hz, CH_2), 1.41 (t, J = 7.4 Hz, CH_3). ^{13}C NMR (acetone-d_6): δ 151.7, 137.7, 135.9, 135.5 (q, J = 34 Hz), 127.7, 122.4, 123.4 (q, J = 270 Hz), 112.2, 101.2, 52.5, 8.0.

Ethiprole Amide (E−Amide) [5-Amino-3-carbamyl-1-(2,6-dichloro-4-trifluoromethylphenyl)-4-ethylsulfinylpyrazole]. Following a general procedure for nitrile hydrolysis (*24*) with slight modification, E−SOC_2H_5 (32 mg, 0.80 μmol) was stirred with ethanol (400 μL) and 50% aqueous sodium hydroxide (400 μL). Water was added to this mixture until it became clear (∼400 μL). After the reaction was stirred for 4 h at 25 °C, it was quenched with saturated ammonium chloride and extracted with ethyl acetate three times. Purification of the brown residue by flash chromatography with ethyl acetate/hexane (1:1) followed by ethyl acetate/hexane (3:1) provided E−amide (23 mg, 72%); mp 176−180 °C (dec). ^{1}H NMR (acetone-d_6): δ 8.09 (s, 2× aryl-H), 7.21 (s, CONH), 6.69 (s, CONH), 6.12 (s, NH_2), 3.09 (q, J = 7.4 Hz, CH_2), 1.30 (t, J = 7.4 Hz, CH_3). ^{13}C NMR (acetone-d_6): δ 163.7, 152.5, 145.4, 137.9, 137.1, 134.6, (q, J = 34 Hz), 127.3, 123.5 (q, J = 270 Hz), 96.5, 48.9, 5.3.

Photodecomposition. Two studies were made comparing ethiprole and fipronil as thin films (0.25 μg/cm^2) in Petri dishes (5 cm diameter) exposed to direct sunlight in Berkeley, CA. The first investigation considering relative rates of photodecomposition was in June with exposure for 0, 1, 3, 6, and 12 h at 25−30 °C. The residue in each dish was dissolved in acetonitrile/water (1:1) (1 mL) for HPLC analysis with quantitation relative to recovery of the parent ethiprole or fipronil at zero time. A control kept in the dark for 30 h gave >93% recovery of the parent compound and no indication of degradation products. The second study for residue characterization was in October over several days with less intense light and 29 h of sunlight exposure for ethiprole and 16 h of sunlight for fipronil at 20−25 °C with analysis by dissolving the residue in acetone (1 mL) for GC/MS identification of the photoproducts.

Metabolism by P450. Human recombinant CYP3A4 (coexpressed with P450 reductase and cytochrome b5) and pooled human microsomes were from Gentest (Woburn, MA). Human CYP3A4 was used because it is the principal isoform for xenobiotic metabolism (*25*). Each incubation mixture contained CYP3A4 (200 pmol of P450, 0.37 mg of protein) or pooled microsomes (21 pmol of P450, 1 mg of protein) and NADPH (1.5 mM, final concentration) in 100 mM potassium phosphate pH 7.4 buffer adding the substrate (2.5 μg) last as a solution in acetone (2.5 μL). Incubations in a final volume of 160 μL were in plastic Eppendorf tubes (500 μL) for 30 min at 37 °C unless indicated otherwise. The reaction was stopped by extraction with ethyl acetate (200 μL) and centrifugation (10 000*g*, 5 min). The organic phase was dried (sodium sulfate) and subjected to GC/MS analysis. As controls, ethiprole and fipronil were stable on incubation with enzyme but no NADPH for 30 min at 37 °C. Quantitation of the sulfone metabolites was relative to standard curves for the authentic compounds.

Metabolites in Mouse Brain and Liver. Male albino Swiss−Webster mice (23−26 g) from Harlan Laboratories (Indianapolis, IN) were treated i.p. with ethiprole or fipronil at 30 mg/kg using Me_2SO (60 μL) as the carrier vehicle. Mice were sacrificed by cervical dislocation after 0.25, 0.5, 1, 2, or 4 h, and the brain and liver were collected for immediate analysis. In another study, the P450 inhibitor PB was administered i.p. at 0 (control) or 80 mg/kg with Me_2SO as the carrier vehicle 40 min before ethiprole at 30 mg/kg. The poisoning signs (if any) were observed at 2 h followed by sacrifice of the mice and recovery of the brain and liver. Each tissue was homogenized using a glass-Teflon homogenizer in 10 mM phosphate buffer (pH 7.4) (5 mL), which was then extracted with ethyl acetate (5 mL). The organic phase was recovered following centrifugation, washed with water (1 mL), and dried (sodium sulfate). The extract was then evaporated to dryness, and the residue was redissolved in water/acetonitrile (1:1) (500 μL) for HPLC analysis of a 100 μL aliquot. Quantitation involved comparison with standard curves for the sulfoxides and sulfones. For recovery analyses, samples of mouse brain and liver were fortified with ethiprole or fipronil at levels ranging from 0.1 to 50 ppm. They were homogenized in 10 mM pH 7.4 phosphate buffer and the homogenate was allowed to settle for 30 min prior to extraction and processing as above. The recovery values for each compound were >90% for brain and >95% for liver.

[^{3}H]EBOB Binding Assay. Two GABA receptor preparations were used to compare a mammalian with an insect source. Human homomeric β3 receptors expressed in Sf9 cells (*17*, *18*) were recovered by

homogenizing the cells in 10 mM phosphate buffer (pH 7.5) containing 300 mM sodium chloride and centrifugation (removal of cellular debris at 500*g* for 10 min and then pelleting the membranes at 100 000*g* for 40 min at 4 °C). House fly heads were collected and homogenized in 0.25 M sucrose and 10 mM Tris-hydrochloric acid buffer (pH 7.5) (*16*) using a Polytron Homogenizer PT 10/35 (Brinkman, Westbury, NY) with a 30 s burst and then a 60 s pause three times in sequence. The homogenate was filtered through four layers of cheesecloth and centrifuged at 1500*g* for 10 min, and the supernatant was filtered again and centrifuged at 23 000*g* for 25 min. The pellet was resuspended in the sucrose−Tris-hydrochloric acid buffer, maintained at 5 °C for 25 min (*26*), and centrifuged at 23 000*g* for 25 min. Finally, the pellet was suspended in 10 mM sodium phosphate buffer (pH 7.5) containing 300 mM sodium chloride and used for binding assay. Protein was determined by the method of Lowry et al. (*27*) with bovine serum albumin as the standard.

The assay mixture was prepared by sequential addition of phosphate buffer−sodium chloride as above (385 μL), the candidate inhibitor in Me_2SO (5 μL), [^{3}H]EBOB (final concentration 0.8 nM) in ethanol/water (1:20) (10 μL), and membrane preparation (100−200 μg of protein) in phosphate buffer−sodium chloride (100 μL). Following incubation with mild shaking for 70 min at 25 °C, the samples were filtered through GF/B paper (Brandel, Gaithersburg, MD) [presoaked in 0.1% polyethylenimine (v/v) for 1 h] and rinsed three times with 5 mL of ice cold rinsing buffer (0.9% sodium chloride w/v) using a Brandel 24 well cell harvester. Radioactivity bound to membranes on the filters was measured with a scintillation counter using OptiPhase "HiSafe" 2 liquid scintillation counting solution (2 mL) (Wallac Oy, Turku, Finland) after standing overnight at room temperature. Nonspecific binding was determined in the presence of 0.5 μM α-endosulfan. Each experiment was repeated three or four times with duplicate analyses. Sigma Plot version 4.01 (SPSS Inc., Chicago IL) was used for least-squares regression analysis to determine the concentration for 50% inhibition (IC_{50} value).

Toxicity to House Flies. Topical LD_{50} values were determined for adult female house flies (SCR susceptible stain) 24 h after topical application of the test compound in 0.5 μL of acetone to the ventrum of the abdomen. For synergism studies, PB was applied topically to the thorax at 250 μg/g 2 h before the test compound because under these conditions it would inhibit most P450-catalyzed oxidative detoxification (*8*). The insecticides were applied in a 1, 3, 10, 30 etc. dose series with 10 house flies per group and three separate experiments to derive the LD_{50} values from log dose−probit mortality plots.

RESULTS

GC/MS and HPLC Characteristics (Table 1). The GC conditions of a 200 °C injector temperature and 60−250 °C column temperature program over 19 min were selected to avoid thermal decomposition of $E-SOC_2H_5$, which occurred much more readily than that of $F-SOCF_3$. Baseline separations of the ethiprole derivatives were achieved on a 15 m DB5 column and of the fipronil derivatives on a 30 m DB1 column using a different temperature program. The order of elution in the ethiprole derivative series was the same as that in the fipronil series (except for F−H). Common fragments for all compounds in the ethiprole series were $[M]^+$, $[C_7H_2F_3Cl_2]^+$, and $[C_7H_3F_3Cl]^+$ (except E−amide) and another common fragment for the thio compounds $E-SC_2H_5$, $E-SOC_2H_5$, $E-SO_2C_2H_5$, and E−amide was $[C_6H_2(Cl_2)(CF_3)(N_2CH_2]^+$. Fragmentation patterns for the fipronil series were given earlier by Bobé et al. (*9*) and Ngim et al. (*11*). All photoproducts and CYP3A4 metabolites were characterized by GC cochromatography and identical MS spectra to those of the authentic standards.

HPLC provided convenient baseline separation of all of the ethiprole derivatives. The photoproducts and metabolites were identified by comparison of retention time (t_R) values and UV spectra with synthetic standards.

Photodecomposition. The rates of photodecomposition were compared for ethiprole and fipronil exposed to sunlight as thin films on glass surfaces with analysis by HPLC (**Figure 3**). The rate of ethiprole loss was somewhat less than that of fipronil, approximating first-order kinetics in each case ($r^2 = 0.98$ in both cases) and half-time ($t_{1/2}$) values of about 14 and 6 h, respectively (**Figure 3**).

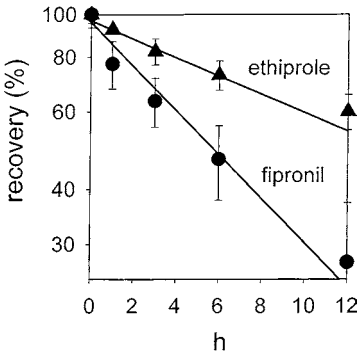


Figure 3. Photodecomposition rates for ethiprole and fipronil exposed to sunlight as thin films on glass surfaces. Data points and bars are means ± SE ($n = 3$). h represents cumulative sunlight exposure.

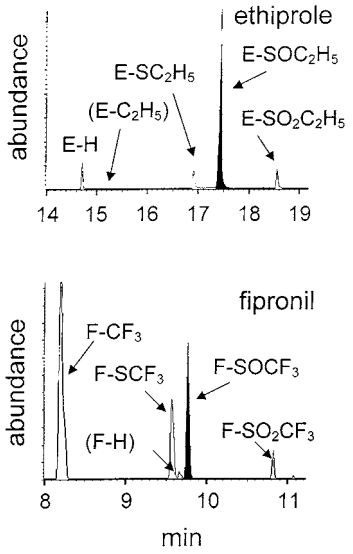


Figure 4. Representative GC/MS chromatograms for ethiprole and fipronil and their photoproducts exposed to sunlight as thin films for 29 and 16 h, respectively. Analysis involved different conditions for ethiprole and fipronil (**Table 1**). Compounds in parentheses shown by chromatographic position but not evident as photoproducts are $E-C_2H_5$ (t_R 15.18 min) and F–H (trace amount as impurity in fipronil) (t_R 9.67 min). Ethiprole amide (t_R 19.48 min) and fipronil amide (t_R 12.44 min) were not observed under the test conditions.

The pattern of photoproducts was quite different for ethiprole and fipronil based on GC/MS analyses (**Figure 4**). The exposure time was longer for ethiprole than fipronil because it photodecomposes more slowly (**Figure 3** and also confirmed by HPLC analysis of the experiment shown in **Figure 4**). Ethiprole gave three significant photoproducts at 29 h identified by GC/MS comparison with authentic standards: $E-SO_2C_2H_5$ from oxidation, $E-SC_2H_5$ from reduction, and E−H from desethylsulfinylation. Interestingly, no $E-C_2H_5$ was detected from desulfinylation. Fipronil at 16 h exposure gave the expected $F-SCF_3$ and $F-SO_2CF_3$ and no F−H, but the principal product was $F-CF_3$ from desulfinylation. Thus, the major photoproducts, other than the sulfides and sulfones, were E−H with ethiprole and $F-CF_3$ with fipronil (**Figure 4**). This was not only the case for thin films exposed to sunlight but also for solutions in

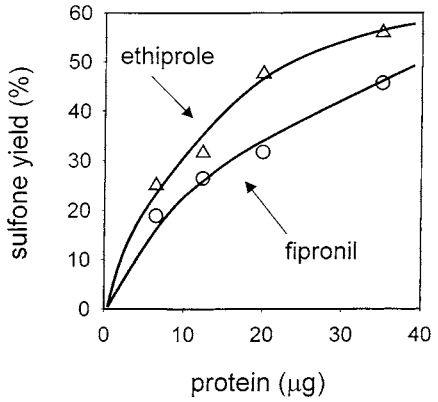


Figure 5. Oxidation of ethiprole and fipronil to the corresponding sulfone by human expressed CYP3A4 with NADPH.

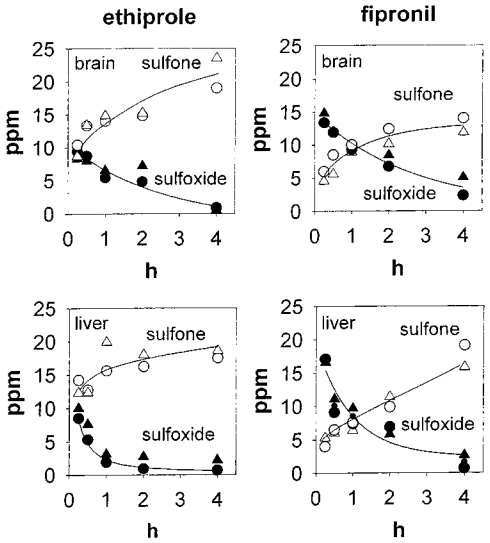


Figure 6. Ethiprole and fipronil and their sulfone metabolites in the brain and liver of mice as a function of time after i.p. administration of the sulfoxide at 30 mg/kg. Data for two experiments are shown as individual animals at each time point, i.e., ○ and △ for sulfoxide and ● and ▲ for sulfone.

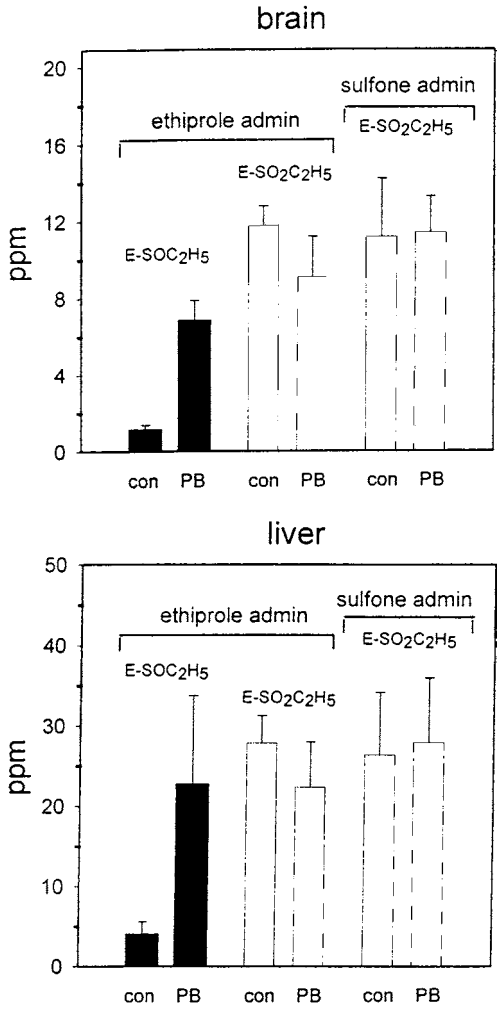


Figure 7. Effect of PB on ethiprole conversion to its sulfone metabolite in brain and liver and on poisoning signs in mice. PB administered i.p. at 0 (control) or 80 mg/kg 40 min before ethiprole or the sulfone at 30 mg/kg. Brain and liver levels determined 2 h after ethiprole or sulfone administration. Only two groups showed poisoning signs: ethiprole with PB, severe convulsions for all five mice; ethiprole control (no PB), less severe convulsions and for only 2 of 5 mice. Data are means ± SD (n = 5).

anhydrous methanol or 1% water in methanol irradiated for 2 and 8 h at 254 nm (data not shown).

Metabolism. *1. General.* Three types of studies were made. The first used human recombinant CYP3A4 to determine possible NADPH-dependent product formation, identify the product(s), and compare the ease of metabolism of ethiprole and fipronil. The second considered the in vivo metabolites in brain and liver of i.p.-treated mice for ethiprole vs fipronil as a function of time. Finally, the effect of the P450 inhibitor PB was determined on the levels of metabolites of ethiprole and its sulfone in brain and liver in relation to the poisoning signs.

*2. Oxidation of Ethiprole and Fipronil by CYP3A4 (**Figure 5**).* Ethiprole and fipronil were converted to the corresponding sulfones on incubation with CYP3A4 and NADPH; no other metabolites were observed. Ethiprole was oxidized a little faster than fipronil on varying the protein level (**Figure 5**) and incubation time (8−30 min, data not shown). Human liver microsomes also converted ethiprole and fipronil to the corresponding sulfones, in each case only on fortification with NADPH (data not shown).

3. Brain and Liver Levels of Ethiprole and Fipronil and Their Sulfone Metabolites in Mice. Ethiprole and fipronil were compared under identical conditions for distribution and fate in i.p.-treated mice using HPLC analysis (**Figure 6**). Two compounds were observed in brain and liver in each case, the parent sulfoxide and the sulfone metabolite. The levels of sulfoxide and sulfone combined were maintained at 20−25 ppm in both tissues at 0.25−4 h posttreatment. The sulfone was formed more rapidly with ethiprole than fipronil and along with the parent compound was essentially the only phenylpyrazole-derived compound in the brain at 4 h after treatment. The ethiprole and fipronil entering the brain within a few minutes after treatment were largely and probably locally converted to the corresponding sulfone in the next 2−4 h.

*4. Effect of PB on Ethiprole Conversion to its Sulfone Metabolite and on Poisoning Signs in Mice (**Figure 7**).* The in vivo conversion of ethiprole (30 mg/kg) to the sulfone (2 h posttreatment) was partially blocked by PB in both brain and liver. On administering the sulfone directly, its level in brain and liver was not affected by PB. Trace levels of ethiprole were detected in the liver but not the brain following sulfone administration suggesting a possible reductive pathway. The

Table 2. Potency of Ethiprole, Fipronil, and Their Derivatives as Inhibitors of [^{3}H]EBOB Binding to House Fly and Human β3 GABA Receptors

	IC_{50} (nM, mean ± SE, $n = 3$ or 4)	
compd	house fly	human β3
	ethiprole series	
E–H	123 ± 35	135 ± 25
E–C_2H_5	13 ± 3	5.1 ± 2.4
E–SC_2H_5	0.7 ± 0.5	0.5 ± 0.1
E–SOC_2H_5	15 ± 3	12 ± 2
E–$SO_2C_2H_5$	20 ± 11	7.8 ± 3.2
	fipronil series	
F–CF_3	13 ± 5	3.2 ± 1.8
F–SCF_3	3.0 ± 0.6	6.4 ± 1.6
F–$SOCF_3$	2.3 ± 0.7	3.1 ± 0.6
F–SO_2CF_3	7.1 ± 3.1	8.4 ± 2.4

Table 3. Toxicity of Ethiprole, Fipronil, and Their Derivatives to House Flies Alone and with PB

	LD_{50} (μg/g, mean ± SE, $n = 3$)	
compd	alone	PB[a]
	ethiprole series	
E–H	>150	8.5 ± 0.5
E–C_2H_5	0.69 ± 0.06	0.15 ± 0.03
E–SC_2H_5	0.70 ± 0.05	0.22 ± 0.03
E–SOC_2H_5	0.50 ± 0.03	0.30 ± 0.02
E–$SO_2C_2H_5$	0.25 ± 0.05	0.13 ± 0.01
	fipronil series	
F–CF_3	0.053 ± 0.005	0.045 ± 0.004
F–SCF_3	1.05 ± 0.05	0.15 ± 0.02
F–$SOCF_3$	0.16 ± 0.01	0.023 ± 0.004
F–SO_2CF_3	0.15 ± 0.01	0.045 ± 0.003

[a] PB applied at 250 μg/g for 2 h before the test compound.

poisoning signs of ethiprole were increased by PB and were greater than that of the sulfone alone or with PB (**Figure 7** legend).

Biological Activity. *1. Potency as Inhibitors of [^{3}H]EBOB Binding (**Table 2**).* The studies compared the potency of ethiprole, fipronil, and their derivatives as inhibitors of [^{3}H]-EBOB binding to house fly and human β3 GABA recptors. The house fly and β3 receptors were overall of similar sensitivity to the compounds examined with average IC_{50} values ($n = 9$) of 22 and 20 nM, respectively. E−H (IC_{50} 123−135 nM) and E−SC_2H_5 (IC_{50} 0.5−0.7 nM) were clearly less and more potent, respectively, than the other phenylpyrazoles. Potency values for the remaining compounds (IC_{50} 2−20 nM) did not follow a discernible structure−activity pattern.

*2. Toxicity to House Flies Alone and with PB (**Table 3**).* The most toxic compound was F−CF_3 (LD_{50} 0.053 μg/g) followed by F−$SOCF_3$, F−SO_2CF_3, and E−$SO_2C_2H_5$ (LD_{50} 0.15−0.25 μg/g). The ethiprole series (sulfide, sulfoxide, and sulfone) was equitoxic with the corresponding fipronil series when comparing the average of the LD_{50} values (0.48 and 0.45 μg/g, respectively). High toxicity was retained for E−C_2H_5 but not E−H. Synergism of the sulfides, sulfoxides, and sulfones was greater in the fipronil than the ethiprole series (6- vs 2-fold, respectively) and the least for F−CF_3. The site(s) of oxidative detoxification blocked by PB were not established.

DISCUSSION

Ethiprole vs Fipronil. An electron-donating ethylsulfinyl group in ethiprole replaces the strongly electron-withdrawing trifluoromethylsulfinyl substituent of fipronil. Ethiprole is also much less lipophilic than fipronil. Examples of this include (i) silica gel TLC R_f values of 0.14 for ethiprole and 0.61 for fipronil developed with ethyl acetate−hexane (1:1); (ii) t_R values of 10.0 min for ethiprole and 18.5 min for fipronil on reverse phase HPLC with acetonitrile/water (1:1); and (iii) log *P* values of 2.6 for ethiprole and 3.8 for fipronil as found on Scifinder Scholar (American Chemical Society) and calculated using Advanced Chemistry Development (ACD) Software Solaris V4.67 (1994−2003 ACD). The present investigation uses standard assay systems in comparing the ethiprole and fipronil series as to their photochemistry, metabolism, and biological activity.

Photochemistry. Fipronil as noted before (*7*) and ethiprole observed here both undergo photochemical oxidation (major) and reduction (minor). Aromatic sulfoxides are known to undergo both unimolecular and bimolecular photochemical deoxygenation (*28*). Under the thin film conditions used in the present study, the main reaction is desethylsulfinylation for ethiprole to give E−H and desulfinylation for fipronil to give F−CF_3 (**Figure 2**). Importantly, there is no desulfinylation of ethiprole to E−C_2H_5. Photochemical cleavage reactions of other sulfoxides (*11*, *29*, *30*) are consistent with ethiprole undergoing primarily homolytic cleavage of the pyrazole−SOC_2H_5 bond to give E−H whereas fipronil undergoes concerted SO extrusion to give F−CF_3.

Metabolism. The only metabolic reaction observed in the human recombinant CYP3A4 and pooled microsomal systems and in vivo in mice is the oxidation of the sulfoxide to the sulfone. If other metabolites are formed, they are not detected by the HPLC or GC/MS systems used. The sulfoxide moiety of ethiprole is always more easily oxidized than that of fipronil. This conversion of ethiprole to the sulfone in mice is partially blocked by PB as noted earlier with fipronil (*8*).

GABAergic Action. The phenylpyrazoles are more potent inhibitors of [^{3}H]EBOB binding with membranes from house fly head than from human brain (*12*, *17*) with IC_{50} values of 15 and 2.3 nM for E−SOC_2H_5 and F−$SOCF_3$ with house fly (this study) and >10 000 and 2470 nM for E−SOC_2H_5 and F−$SOCF_3$, respectively, with human brain membranes (*17*, *18*). In contrast, the house fly receptor is very similar to the human β3 homomeric receptor in [^{3}H]EBOB, ethiprole, and fipronil binding, i.e., the native mammalian receptor selectivity is attributable to its subunit composition and organization (*17*, *18*). The present study illustrates the intrinsic similarity between the house fly and the human β3 receptors and relatively small effect of the sulfide, sulfoxide, and sulfone substituents. It is a further example with phenylpyrazoles of the importance of receptor subunit modulation in conferring compound-dependent specificity and selective toxicity.

Insecticidal Activity. The new studies are based on house flies, topical application, and the effect of PB. Within these limits, there are only moderate substituent effects (C_2H_5 vs CF_3 and S vs SO vs SO_2) on potency although E−H is of much lower activity. PB synergism is considerably lower for the ethiprole than the fipronil series (except E−H and F−CF_3) indicating the greater importance of oxidative detoxification for fipronil and its sulfide and sulfone. All of the compounds except E−H are highly potent in both the house fly [^{3}H]EBOB binding assays and the PB-synergized toxicity relating the receptor potency and overall toxicity.

Mammalian Toxicity. The mouse i.p. acute toxicity of ethiprole is reduced on conversion to E−$SO_2C_2H_5$ (this study) and E−H (in ref *7* referred to as destrifluoromethylsulfinyl-fipronil) whereas the F−CF_3 photoproduct is more toxic and

persistent than fipronil (*7*, *8*). The acceptable daily intake is set about 7-fold lower for the photoproduct F−CF_3 as compared with fipronil (*4*, *31*). Thus, ethiprole circumvents a question brought up for fipronil (*7*) of the potential toxicity of a photoproduct not formed as a metabolite. These are favorable properties for ethiprole in model systems possibly applicable to actual use conditions.

ABBREVIATIONS USED

[^{3}H]EBOB, 4-*n*-[2,3-$^3H_2$]propyl-4′-ethynylbicycloorthobenzoate; E−SOC_2H_5, ethiprole; F−$SOCF_3$, fipronil; GABA, γ-aminobutyric acid; IC_{50}, concentration for 50% inhibition; PB, piperonyl butoxide; t_R, retention time.

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