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Substituted Thiotrifluoropropanones as Potent Selective Inhibitors of Juvenile Hormone Esterase

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A series of 27 substituted thio-1,1,1-trifluoropropanones was synthesized by reacting the corresponding thiol with 1,1,1-trifluoro-3-bromopropanone. The resulting sulfides were screened as inhibitors of hemolymph juvenile hormone esterase and α-naphthyl acetate esterase activity of the cabbage looper, *Trichoplusia ni*, electric eel acetylcholinesterase, bovine trypsin, and bovine α-chymotrypsin. The presence of the sulfide bond increased the inhibitory potency on all of the enzymes tested when compared with compounds lacking the sulfide. In general, the compounds proved to be poor inhibitors of chymotrypsin and moderate inhibitors of trypsin. By varying the substituent on the sulfide, good inhibitory activity was obtained on α-naphthyl acetate esterase, acetylcholinesterase, while some of the compounds proved to be extremely powerful inhibitors of juvenile hormone esterase. The most powerful inhibitor tested was 3-octylthio-1,1,1-trifluoro-2-propanone, with an I_{50} of $2.3 \times 10^{-9} M$ on JH esterase. This compound showed a molar refractivity similar to that of the JH II backbone, was not toxic to *T. ni*, and was moderately toxic to mice, with a 48-hr LD_{50} of >750 mg/kg. It effectively delayed pupation when applied to prewandering larvae of *T. ni*, as expected for a JH esterase inhibitor. Thus, some members of this series are promising for evaluating the role of JH esterase in insect development. The series also indicates that, by varying the substituent on the sulfide moiety, potent "transition-state" inhibitors can be developed for a wide variety of esterases and proteases.

INTRODUCTION

The inhibitory activity of serine esterases and proteases of the highly polarized trifluoromethylketone moiety has been discussed in two previous manuscripts (1, 2), and the high activity of these compounds appears to be due to their ability to form hemiketals with the serine present at the enzyme catalytic site. Thus, the compounds could be considered as substrate analogs or transition-state analogs based on the definition of Wolfenden (3).

The enzymes, which catalyzed the hydrolysis of the methyl ester of insect juvenile hormone (JH)[3] to yield the inactive metabolite, juvenile hormone acid, are thought to proceed with a change in the bond order of the substrate (trigonal to tetrahedral). Such enzymes are excellent candidates for inhibition by transition-state analogs (4). The juvenile hormone esterase (JHE) activity of the cabbage looper, *Trichoplusia ni* (Lepidoptera, Noctuidae), further appears largely to be due to a single enzyme (5, 6). Thus, it was chosen as a target for the development of transition-state analogs.

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[3] Abbreviations used: JH(s), juvenile hormone(s); TFT, 1,1,1-trifluorotetradecan-2-one; TLC, thin-layer chromatography; GLC, gas–liquid chromatography; ANS, 8-anilino-1-naphthalenesulfonic acid; DCQ, 2,6-dibromo-N-chloro-*p*-benzoquinoneimine; NBP, 4-(4′-nitrobenzyl)-pyridine; ir, infrared spectroscopy; NMR, ^1H nuclear magnetic resonance spectroscopy; BTFA, 3-bromo-1,1,1-trifluoroacetone; PTFP, 3-phenylthio-1,1,1-trifluoro-2-propanone; OTFP, 3-octylthio-1,1,1-trifluoro-2-propanone; α-NA, α-naphthyl acetate; AChE, acetylcholinesterase; α-ChT, α-chymotrypsin; EPPAT, *O*-ethyl-*S*-phenyl phosphoramidothioate; NTFP, 3-nonylthio-1,1,1-trifluoro-2-propanone.

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1,1,1-Trifluorotetradecan-2-one (TFT) was reported previously to be a potent inhibitor of the JH esterase from *T. ni*, with an I_{50} of 1×10^{-7} *M* (2). In spite of its potency, it failed to cause the *in vivo* symptoms expected from complete inhibition of JH esterase. Since JH is an α–β unsaturated ester, a sulfide bond was placed β to the carbonyl in hopes that it would mimic the 2,3-olefin of JH and yield more powerful inhibitors. The inhibitory potency of the resulting compounds was measured on JH esterase as well as on four additional enzymes as an indication of potential selectivity, and compared with previously synthesized compounds. Finally, the *in vivo* action of the best member of the series was examined.

MATERIALS AND METHODS

Analysis and purification. Where appropriate, compounds were purified by distillation, recrystallization, preparative thin-layer chromatography (TLC) on 2000-μm silica gel plates (Analtech), or column chromatography on Florisil (60/100 mesh, Floridin, Co., Berkeley Springs, W. Va.) using a hexane:ether gradient. In most cases the reaction mixture following differential extraction appeared pure except for a minor high R_f (TLC) or short retention time (gas–liquid chromatography, GLC) product which cochromatographed with the starting thiol.

Analytical TLC was performed on silica gel F_{254} plates of 250 μm thickness from EM Laboratories. Aromatic compounds were detected by their quenching of gel fluorescence when exposed to shortwave ultraviolet light (254 nm). Alternatively, all materials could be readily detected by spraying the plates with 8-anilino-1-naphthylenesulfonic acid (ANS, Sigma, 10 mg/100 ml water) and observing the plates under longwave ultraviolet light (365 nm). Spraying the plates with 2,6-dibromo-*N*-chloro-*p*-benzoquinoneimine (7) (DCQ, Eastman–Kodak, 50 mg/100 ml hexane) and subsequent exposure to hydrochloric vapor gave dark-red spots for thiols and disulfides and light-orange or tan spots for the product sulfides. A spray of 4-(4′-nitrobenzyl)pyridine (NBP) followed by heating and spraying with base yielded blue spots in the presence of reactive halogens or reactive alkylating agents such as some epoxides (8). TLC plates were developed in toluene:*n*-propanol, 20:1, or hexane:ether, 5:1, with the latter system giving sharper spots. In all cases the starting material had a higher R_f than the product sulfide, and the corresponding sulfoxide demonstrated a lower R_f. The purity of the products was also evaluated by GLC using a Varian Series 1400 equipped with a flame ionization detector and glass column (2 m × 2 mm i.d.) packed with 5% SE 30 on Gas Chrom Q (air, 300 ml/min; H_2, 20 ml/min; N_2, 15 ml/min).

Gas–liquid chromatography mass spectroscopy (GLC/MS) data were obtained on selected compounds on a Finnigan Model 3200. The compounds were injected in dichloromethane onto an SE 52 or DB-1 30 m × 0.25 mm i.d. column initially at 60°C. The temperature was increased at 6°C per minute, and the total ion current was measured at 70 eV. Mass spectra were taken at 1.9-s intervals. Infrared (ir) spectra were obtained on a Perkin–Elmer Model 334 run as thin films of the respective compound on silver chloride plates. Proton magnetic resonance (^{1}H NMR) spectra were obtained on a Varian EM-390 in a dilute solution of carbon tetrachloride or ^{2}H chloroform with or without deuterium oxide. The compounds were examined in the hydrated form. Tetramethylsilane was used as an internal standard. Melting points reported are uncorrected.

Synthesis. The aliphatic and aromatic trifluoromethylketones were prepared by either a modified Grignard procedure (9, 10) or using iron tetracarbonyl (11, 12) as described earlier (2). In contrast, members of the sulfide series were prepared by the reaction of the appropriate thiol with

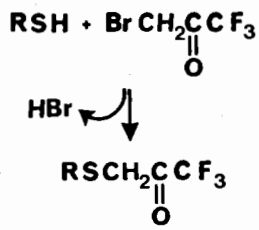


FIG. 1. *Synthesis of trifluoropropanone sulfides (substituted-1,1,1-thiotrifluoro-2-propanones). The appropriate thiol is reacted with 3-bromo-1,1,1-trifluoropropan-2-one.*

3-bromo-1,1,1-trifluoropropan-2-one (3-bromo-1,1,1-trifluoroacetone, BTFA) (PCR Research Chemicals Inc., Gainesville, Fla.) (Fig. 1). Typical syntheses are outlined below.

3 - Benzylthio - 1,1,1 - trifluoro - 2 - propanone. Benzyl mercaptan (4 mmol) was transferred via syringe to a 2-cm-diameter bottle sealed with a rubber septum and containing 2 ml carbon tetrachloride under nitrogen. BTFA (5 mmol) was added dropwise via syringe while the reaction was stirred magnetically. The reaction was allowed to proceed under a gentle stream of nitrogen, and the evolving hydrogen bromide gas was trapped with sodium hydroxide pellets. The reaction was monitored by TLC and the high-R_f starting material was found to vanish rapidly in favor of a single low-R_f spot. After 24 hr 5 ml ethyl ether was added and the reaction mixture was washed twice with 5% w/v aqueous sodium bicarbonate and once with saturated brine. The ether layer was dried (Na_2SO_4), and the solvent was evaporated to give a faintly orange oil in 88% isolated yield, which by GLC and TLC showed only a trace of the starting material. The oil was recrystallized twice from hexane to give colorless, odorless cubes of the benzylsulfide (H, Table 1) in 60% yield. The crystals rapidly turned orange with the release of benzyl thiol upon exposure to air. mp 60–61°C; NMR (CCl_3D) δ 2.93, 3.35, 3.80 (3s, 2, $CH_2C(OH)_2$ or C=O), δ 4.05 (s, 2, CH_2 Ph), δ 7.45 (s, 5, Ph); ir (neet, AgCl) 3500 (br s), 1775 (w), 1750 (s), 1175 (br s).

3 - Phenylthio - 1,1,1 - trifluoro - 2 - propanone. Thiophenol (75 mmol) was distilled under nitrogen into a dry 50-ml flask with a stir bar. Carbon tetrachloride (10 ml) was added, followed by the slow addition of BTFA (75 mmol) while the reaction mixture was held at about 20°C with a water bath. Following the addition of BTFA, the reaction was run as before and appeared to be complete within 4 hr. The reaction mixture was washed as before and dried, and the ether layer was concentrated to give a quantitative recovery of clear oil showing approximately 12% starting material by GLC. The clear, colorless oil was distilled to give the phenylsulfide (abbreviated PTFP) (**I**, Table 1) in 83% isolated yield. Following storage at −5°C, white needles formed in the oil. bp 65.0–65.5°C at 2.9 mm Hg; NMR (CCl_4) δ 3.95 (s, CH_2), δ 7.4 (m, Ph); ir (neet, AgCl) 3500 (br s), 3100 (w), 1775 (w), 1750 (m), 1625 (br w), 1580 (s), 1480 (s), 1440 (s), 1200 (br s) mass spectrum, *m/z* (rel intensity %), 220 M^+ (90), 124 (25), 123 (100), 110 (94), 109 (71), 77 (57), 69 (41), 66 (55), 65 (44), 58 (84). Two other members of the series were purified by crystallization, including the β-naphthyl sulfide (**AA**, Table 1, white plates from ether hexane, mp 133–133.5°C), and the *o*-chlorophenylsulfide (**R**, Table 1, white needles from benzene, mp 64.5–67°C).

3-Octyl-1,1,1-trifluoro-2-propanone. The octylsulfide (abbreviated OTFP) was prepared similarly, except that the starting materials was not distilled. Following workup, the clear oil was distilled to give the product sulfide (**B**, Table 1) in 84% isolated yield (bp 87–89°C at 0.2 mm Hg). IR (neet AgCl) 3400 (br s), 2920 (s), 1770 (m), 1460 (m), 1160 (br s); mass spectrum, *m/z* (rel intensity %) 256 M^+ (5), 199 (2), 171 (1), 145 (100), 159 (30), 129 (15), 69 (45); other members of the series were colorless needles (nonylsulfide and decylsulfide; **C** and **D**, Table 1) or white, waxy solids (cyclohexylsulfide, undecylsulfide, and dodecylsulfide; **G**, **E**, and **F**, Table 1).

Enzyme assays. JH esterase activity was

TABLE 1

Inhibition of JH Esterase, α-Naphthyl Acetate Esterase, Acetylcholinesterase, and Trypsin by Substituted Trifluoropropanone Sulfides

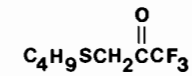
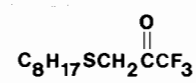
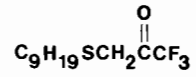
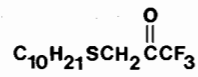
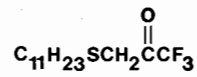
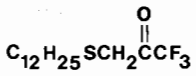
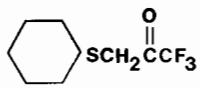
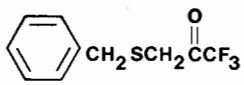
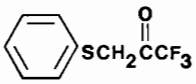
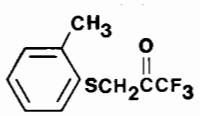
Compound		I_{50} (M)[a] (slope)			
Letter	Structure	JH Esterase	α-NA Esterase	AChE	Trypsin
A		1.5×10^{-6}[b]	—	4.2×10^{-5} (54)	3×10^{-5}[b]
B		2.3×10^{-9} (62)	1.2×10^{-6} (37)	2.3×10^{-6} (61)	$>1 \times 10^{-5}$
C		3.7×10^{-9} (56)	6.3×10^{-6} (38)	9.3×10^{-7} (94)	$>1 \times 10^{-4}$
D		2.0×10^{-8} (123)	1.0×10^{-5} (38)	3.2×10^{-6} (43)	8.2×10^{-5} (65)
E		4.3×10^{-8} (60)	4.5×10^{-5} (41)	2.6×10^{-6} (88)	$>1 \times 10^{-4}$
F		1.5×10^{-8} (52)	9.7×10^{-5} (43)	2.2×10^{-6} (46)	$>1 \times 10^{-4}$
G		5.2×10^{-6} (42)	7.1×10^{-7} (24)	1.8×10^{-6} (52)	$>1 \times 10^{-4}$
H		1.5×10^{-5} (43)	9.1×10^{-6} (25)	$>1 \times 10^{-4}$	$>1 \times 10^{-4}$
I		8.2×10^{-6} (43)	3.0×10^{-6} (26)	3.7×10^{-6} (51)	$>1 \times 10^{-4}$
J		4.3×10^{-6} (50)	6.0×10^{-7} (24)	3.7×10^{-7} (48)	8.5×10^{-5} (85)

TABLE 1—*Continued*

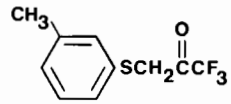
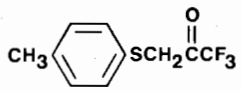
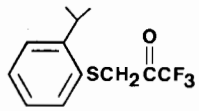
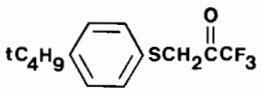
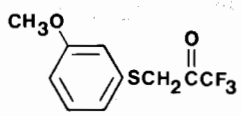
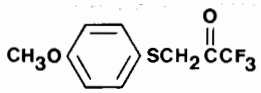
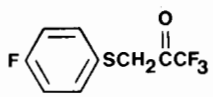
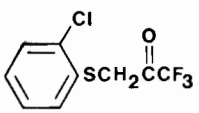
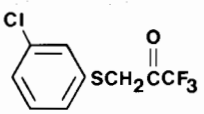
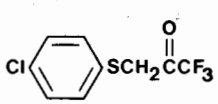
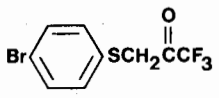
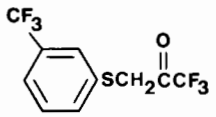
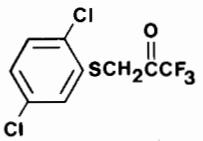
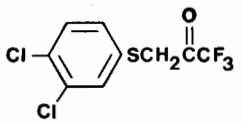
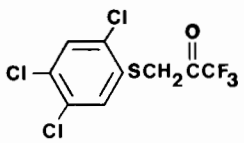
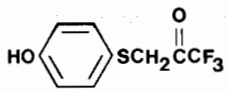
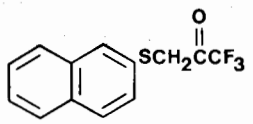
Compound		I_{50} (M)[a] (slope)			
Letter	Structure	JH Esterase	α-NA Esterase	AChE	Trypsin
K		1.3×10^{-7} (21)	1.2×10^{-6} (26)	1.4×10^{-6} (39)	$>1 \times 10^{-4}$
L		1.1×10^{-7} (20)	1.6×10^{-6} (23)	6.1×10^{-7} (41)	$>1 \times 10^{-4}$
M		3.4×10^{-7} (20)	2.7×10^{-7} (26)	4.9×10^{-8} (49)	4.7×10^{-5} (74)
N		7.5×10^{-9} (42)	8.6×10^{-7} (40)	3.2×10^{-6} (64)	—
O		1.3×10^{-6} (32)	7.6×10^{-7} (22)	1.7×10^{-5} (38)	—
P		1.3×10^{-6} (21)	5.5×10^{-6} (33)	7.5×10^{-6} (64)	3.8×10^{-5} (76)
Q		8.4×10^{-6} (40)	1.2×10^{-5} (26)	2.5×10^{-6} (64)	$>1 \times 10^{-4}$
R		2.1×10^{-6} (30)	5.2×10^{-7} (35)	1.2×10^{-6} (62)	—
S		4.0×10^{-7} (27)	7.3×10^{-7} (21)	1.2×10^{-5} (49)	—
T		1.3×10^{-6} (18)	3.5×10^{-6} (43)	1.8×10^{-6} (45)	4.3×10^{-5} (71)

TABLE 1—*Continued*

Compound		I_{50} (M)[a] (slope)			
Letter	Structure	JH Esterase	α-NA Esterase	AChE	Trypsin
U		4.1×10^{-7} (21)	4.9×10^{-6} (23)	2.4×10^{-6} (54)	$>1 \times 10^{-5}$
V		2.5×10^{-6} (38)	1.2×10^{-6} (21)	2.6×10^{-5} (40)	—
W		2.3×10^{-8} (51)	2.5×10^{-7} (43)	1.2×10^{-5} (44)	—
X		2.0×10^{-8} (52)	4.0×10^{-7} (20)	1.6×10^{-5} (44)	—
Y		3.2×10^{-8} (60)	9.8×10^{-7} (39)	6.0×10^{-6} (70)	—
Z		6.1×10^{-6} (29)	8.6×10^{-6} (30)	8.7×10^{-6} (94)	—
AA		$>1 \times 10^{-4}$	$>1 \times 10^{-4}$	$>1 \times 10^{-4}$	$>1 \times 10^{-4}$

[a] I_{50} values were determined from lines with r^2 values greater than 0.98 and usually greater than 0.99, except for the I_{50} of compound K on AChE where $r^2 = 0.87$. No inhibition of αChT was observed with any of the compounds at [I] = 1×10^{-4} M. The substrate concentrations were 5×10^{-6}, 2.3×10^{-4}, 5×10^{-4}, 1×10^{-4}, and 5.8×10^{-5} M, respectively for the five enzymes examined. For JH esterase variability in the calculated I_{50}'s among replicates was ca 7.5%, while variability among the means run on different days was ca 5%. Similar values for α-NA esterase were 5 and 2.5%.

[b] Data from Ref. 2.

monitored in the hemolymph collected from last stadium Day-2 larvae of the cabbage looper, *T. ni*, and was then centrifuged at 1000*g*. The plasma was diluted in sodium phosphate buffer [pH 7.4, 0.08 *M* with 0.01% (w/v) phenylthiourea] and frozen at −60°C until used. Phenylthiourea was used to inhibit tyrosinases and did not inhibit JH esterase activity. Substrate (C10 ^{3}H JH III New England Nuclear and unlabeled *E,E* JH III Calbiochem) in 1 μl of ethanol was added to diluted plasma to give a final concentration of 5×10^{-6} *M* JH III containing ~30,000 dpm/assay (2, 13). α-Naphthyl acetate (α-NA) hydrolysis was monitored as described earlier (2, 5) in the hemolymph of prepupae of *T. ni* (fifth stadium, Day 4), with a final substrate concentration of 2.3×10^{-4} *M*. An assay temperature of 30°C was used for both JH and α-NA esterases.

For acetylcholinesterase (AChE) activity, 20 μl acetylthiocholine iodide (Sigma) in sodium phosphate buffer (0.05 *M*, pH 7.2) was added to Type VI-S electric eel AChE (Sigma; 10 ng, with 2–400 units/mg protein) in 3 ml of the same buffer to give a final substrate concentration of 5×10^{-4} *M*. The assay was run at 37°C as outlined previously (14). For trypsin 1.5 ml *p*-toluenesulfonyl-L-arginine methyl ester (Sigma) in Tris–HCl buffer (pH 8.1, 0.04 *M*, with 0.1 *M* $CaCl_2$) was added to 1.5 ml of the same buffer containing bovine pancreatic type III trypsin (Sigma, 0.24 μg) to give a final substrate concentration of 1×10^{-4} *M*. The assay was performed at 25°C as described previously (2, 15). For α-chymotrypsin activity (α-ChT) 50 μl *p*-nitrophenyltrimethylacetate (Aldrich) in acetonitrile was added to the enzyme solution to give a final substrate concentration of 5.8×10^{-5} *M*. The enzyme solution was prepared immediately before the assay by adding 0.1 ml bovine pancreas Type II α-chymotrypsin (Sigma; 1.7 μg, with 40–50 units/mg protein) in sodium acetate buffer (0.01 *M*, pH 4.6) to 2.85 ml sodium phosphate buffer (0.01 *M*, pH 8.0) at 25°C (16).

For JH esterase, α-NA esterase, AChE, trypsin, and α-ChT, the inhibitor was added in ethanol 10 min prior to the substrate and preincubated at the same temperature used for the actual assay. In no case was greater than 1% (v/v) ethanol added, and ethanol controls were always run. Enzyme concentrations and incubation times were selected to give linear hydrolysis rates with time. For the four spectrophotometric enzyme assays initial studies were run on a Varian–Cary 219 uv/visible spectrophotometer, while subsequent assays were run on a Gilford manual EIA reader using disposable cuvettes.

At least three replicates of each inhibitor concentration was used. A minimum of two inhibitor concentrations showing greater and two showing less than 50% inhibition on the linear region of the curve were used to determine the I_{50} values from semilog plots, except in the case of weak inhibitors (Tables 1 and 2, Fig. 2). Only those points on the apparently linear portion of the

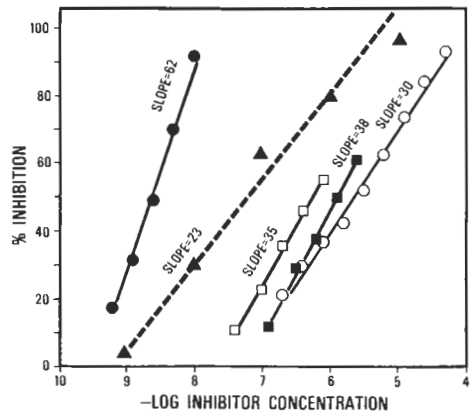


FIG. 2. *Inhibition of JH esterase and α-naphthyl acetate esterase by two substituted thiotrifluoropropanones and TFT. OTFP inhibition of JH esterase* (●); *OTFP inhibition of α-naphthyl acetate esterase* (■); *3-(2-chlorophenylthio)-1,1,1-trifluoro-2-propanone inhibition of JH esterase* (○); *3-(2-chlorophenylthio)-1,1,1-trifluoro-2-propanone inhibition of α-naphthyl acetate esterase* (□); *and TFT inhibition of JH esterase* (▲). *Note that the later compound lacks the sulfide bond and that the inhibition curve has a much lower slope than OTFP. Each inhibitor concentration was run at least three times, and the I_{50} was determined on two or more separate occasions. The r^2 values are >0.98.*

curve were used to calculate the I_{50}s, the slopes, and r^2 values by least-squares regression of the lines, as indicated for selected compounds in Fig. 2.

In vivo activity on T. ni. Larvae were treated with 1 or 2 μl ethanol or with OTFP, TFT, or *O*-ethyl-*S*-phenyl phosphormidothioate (EPPAT) in 1×10^{-1} *M* ethanol solutions on the dorsum of the thorax three times per day (4, 12, and 17 hr after lights on) during the first 2 days of the last larval stadium. The insects were held under standard rearing conditions (27°C; 14 hr light, 10 hr dark). The time of pupation was checked daily at 4 hr after lights on, and the pupae were checked for successful emergence 2 weeks later.

In vivo toxicity to mice. The acute toxicity of OTFP was evaluated by first diluting it appropriately in dimethyl sulfoxide. A 50-μl aliquot of the resulting solution was injected intraperitoneally into male Swiss–Webster mice (25–30 g) to give single 250-, 500-, and 750-mg/kg doses. The mice were observed continually for the first 6 hr; then mortality and symptoms were recorded at 12, 24, 48, and 72 hr. The treated mice were observed for 3 additional weeks.

RESULTS AND DISCUSSION

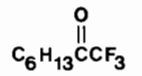
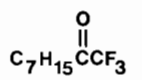
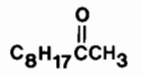
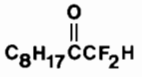
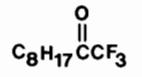
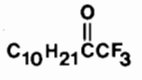
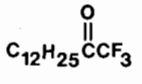
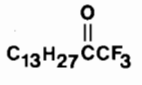
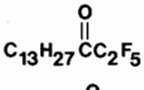
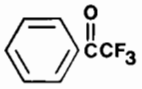
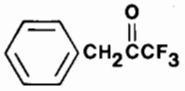
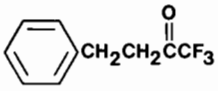
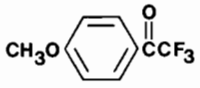
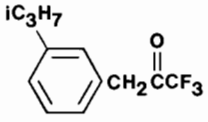
Synthesis

Numerous reaction conditions were evaluated, including several solvents and bases. The procedure in which carbon tetrachloride was used as solvent, with the reaction being driven by the evolution of hydrogen bromide, gave consistently high yields in those cases in which the starting materials were soluble (Fig. 1). Alternatively, benzene (caution, suspect carcinogen) could be used if the addition of BTFA was followed by the very slow addition of triethylamine. This procedure resulted in only slightly lower yields. However, significantly reduced yields were obtained when pyridine or triethylamine were used as solvents, when equimolar amounts of these bases were added to benzene or carbon tetrachloride before the addition of the BTFA, or when the sodium salt of the thiol was exposed to BTFA in ethylene glycol monomethyl ether. The alternative procedure of using methanol as the solvent with sodium bicarbonate as the base also gave consistently high yields, and offered some advantages when working with compounds that were poorly soluble in carbon tetrachloride. This procedure was also used successfully with ethylene glycol monomethyl ether and methylene chloride as solvents. Preparation of PTFP (I) in carbon tetrachloride and in methanol resulted in compounds with apparently identical properties on TLC, ir, and GLC, and indistinguishable slopes and I_{50}s when tested as inhibitors of JH esterase. Cooling seemed to not influence the reactions run on scales of 10 mmol or smaller; however, water baths and slow addition of BTFA were important on larger-scale reactions. Subambient temperatures proved counterproductive because the reactions failed to proceed until they were allowed to warm (17–19). The compounds (A through AA, Table 1) appeared reasonably stable. However, the benzyl sulfide (H) tended to decompose to yield benzyl thiol (by TLC) when allowed to stand at room temperature. In general, the compounds appeared more stable when they contained a trace of starting material.

The compounds demostrated a single major spot on TLC (with shortwave ultraviolet or ANS detection as appropriate) in several systems, with a small, high-R_f spot due to the corresponding thiol. The DCQ reacted strongly with thiol and weakly with the sulfide. Neither spot reacted with NBP, which also indicated the absence of BTFA. BTFA gives a deep-blue color with NBP. Similarly, on GLC a minor, fast-eluting peak was confirmed as the mercaptan based on its mass spectrum, while the only other peak detected on temperature programming to 285°C was the sulfide, which eluted as a sharp, symmetrical peak. OTFP (B) was partially resolved on a DB-1 30-m capillary column into two peaks, with the first peak accounting for 35% of the total

TABLE 2

Inhibition of α-Naphthyl Acetate Esterase and Acetylcholine Esterase by Trifluoroketones Lacking the Sulfide Moiety

Compound		I_{50} (M)[a] (slope)	
Letter	Structure	α-NA Esterase	AChE
I		—	1.5×10^{-5} (37)
II		6.1×10^{-5} (38)	2.2×10^{-5} (47)
III		$>1 \times 10^{-4}$ (0%)[b]	$>1 \times 10^{-4}$ (0%)[b] (NA)
IV		$>1 \times 10^{-4}$ (28%)[b]	2.2×10^{-4} (49)
V		2.7×10^{-5} (32)	1.2×10^{-5} (31)
VI		3.2×10^{-5} (39)	7.1×10^{-5} (42)
VII		$>1 \times 10^{-4}$ (39%)[b]	2.5×10^{-4} (51)
VIII		$>1 \times 10^{-4}$ (37%)[b]	4.3×10^{-5} (74)
IX		$>1 \times 10^{-4}$ (17%)[b]	1.5×10^{-5} (111)
X		—	1.7×10^{-4} (46)
XI		$>1 \times 10^{-4}$ (16%)[b]	1.8×10^{-4} (64)
XII		$>1 \times 10^{-4}$ (39%)[b]	3.0×10^{-5} (45)
XIII		1×10^{-4} (23%)[b]	1.1×10^{-5} (39)
XIV		1×10^{-4} (31%)[b]	2.3×10^{-4} (54)

[a] I_{50} values were determined from lines with r^2 values greater than 0.95 and usually greater than 0.99, except for the I_{50}s of compounds V and VII on AChE, with r^2 values of 0.87 and 0.83, respectively. The substrate concentrations were 2.3×10^{-4} and 5×10^{-4} M, respectively. Inhibitory activity on JH esterase and trypsin has been reported previously (2).

[b] When the I_{50} is $>1 \times 10^{-4}$ M, the percentage inhibition at 1×10^{-4} M is indicated in parentheses.

ion current. The mass spectra obtained throughout the elution of the two peaks appeared identical. A peak at *m/e* 69 was typical of trifluoromethyl compounds, and the base peak was usually attributed to cleavage of the sulfide bond. All spectra showed a clear parent ion.

The infrared spectra also demonstrated both 3400- and 1700-cm^{-1} bands, indicating that the carbonyl was partially hydrated. A strong band at 1150–1200 cm^{-1} was indicative of the trifluoromethyl moiety (20). The NMR spectra were complex probably due, in part, to the compounds being partially hydrated. The spectra of anhydrous materials could be greatly simplified, and $^4J_{HF}$ was only observed in very dry samples (17).

I_{50}s on JH esterase of OTFP (B) and PTFP (I) were tested following synthesis on methanol–bicarbonate and carbon tetrachloride, before and after preparative TLC, and after distillation, and found to be identical. BTFA, BTFA following exposure to bicarbonate, 10 of the thiols used to synthesize the compounds listed in Table 1, and diphenyldisulfide isolated as a decomposition product of the peracid sulfoxidation of the phenylsulfide gave no detectable inhibition of JH esterase at $1 \times 10^{-4} M$. BTFA has been used to derivatize cysteine residues in proteins (17, 21, 22). Since other sulfhydryl reagents have little effect on JH esterase activity, the lack of inhibitory activity by BTFA is not surprising. The I_{50} of 3-nonylthio-1,1,1-trifluoro-2-propanone (NTFP) (C) was identical when exposed to JH esterase in normal buffer or in buffer containing $1 \times 10^{-5} M$ hydrogen peroxide (Roe, unpublished; JH esterase is very stable to peroxide). Alternatively, NTFP at several concentrations from 1×10^{-3} to $1 \times 10^{-5} M$ was exposed to 9 *M* hydrogen peroxide from 0 to 40 min at 30°C or 24 hr at 4°C, and then assayed without work-up as a JH esterase inhibitor. No effect of hydrogen peroxide exposure on inhibitory activity was noted. The corresponding sulfoxide from peracid oxidation of PTFP has a low R_f in the TLC systems used. Therefore, it was possible to verify that compounds had negligible levels of the corresponding sulfoxides. Based on the above information, it seems unlikely that the inhibitory activity of these compounds can be attributed to impurities.

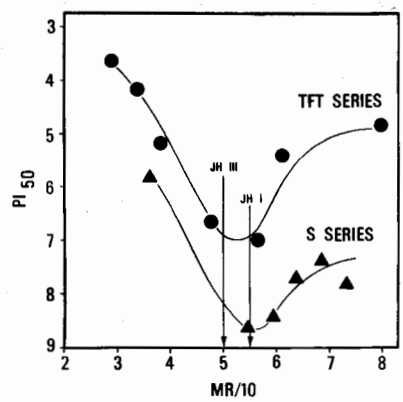


FIG. 3. *Relationship between the log molar concentration of trifluoroketones and trifluoropropanone sulfides required for 50% inhibition of JH esterase from T. ni and the molar equivalents (MR) of R- groups. The arrows show calculated MR values for the backbone of JH III and JH I. The upper curve (●) represents data from the aliphatic trifluoroketone series (Ref. (2)) and the lower curve (▲) represents data from the 3-alkylthio-1,1,1-trifluoro-2-propanone series. The most active compounds are TFT (upper curve) and OTFP (lower curve).*

Structure Activity Relationships

Among the alkylthio trifluoropropanones (A–F, Table 1) there is a clear correlation between the structure and the molar I_{50} values for JH esterase. It is also interesting that the alkyl trifluoromethylketones (TFT homologs) discussed in a previous manuscript (2) demonstrate similar structure activity relationships (Fig. 3). Using molar refractivity (MR) as a physicochemical parameter for R groups in the general structure $RC(O)CF_3$, it is clear that both series show an optimum MR value for strong inhibitory activity, and that activity decreases when MR values are either larger or smaller than this value. As the experimental values of MR are obtained from the expression $MR = [(n^2-1)/(n^2+2)](M_r/d)$,

where N = refractive index, M_r = molecular weight, and d = density (23), it is connected to "bulk" effects in molecular interactions. MR values for the TFT series and JH I and JH III were calculated from the additivitiy principle considering C = 2.418, H = 1.1, ethereal oxygen = 1.6443, and an increment for C=C double bond of 1.733. The same additivity principle was used for the sulfur series, except that the experimental MR value for C_4H_9S-(31.5) as quoted by Balaban *et al.* (24) was used as a basic group. Therefore, it can be concluded from Fig. 3 that the affinity of these compounds for the enzyme active center is a linearly decreasing function of the sum of nonoverlappable volumes of the inhibitor molecule and a receptor cavity on the enzyme active site. Although both series seem to have similar optimum MRs (ca 5.5), the alkyl homologs (such as TFT, Table 2) are less active than the corresponding sulfide analogs (such as OTFP, Table 1). It is worth noting that the calculated MR values for the corresponding straight chain of the juvenile hormones are 4.71 and 5.2, respectively, for JH III and JH O, JH I and JH II. Considering the "optical exaltation" due to the conjugated double bonds in the open chain of JH molecules as 0.285 (0.1 ΔMR between the enol and keto forms of ethyl acetoacetate (25)), the corrected MR values would be 5.00 and 5.49, respectively, for the staight chain of JH III and JH I. These data indicate that JH I and II, rather than JH III, demonstrate the optimum volume to interact with JH esterase based on the structure–activity relationships shown in Fig. 3. Similar relationships are also evident when a variety of steric parameters are used to develop the structure activity relationship illustrated with MR in Fig. 3 (such as chain length or Exner's molar volume).

JH II and I appear to be the major JHs of lepidopterous larvae (Baker and Schooley, personal communication). Since JH esterase from *T. ni* and larvae from several other species of Lepidoptera under

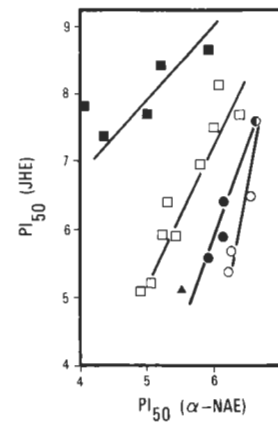


FIG. 4. *Correlation between inhibition of JH esterase and α-naphthyl acetate esterase. The lines represent the aliphatic series (■), the unsubstituted aromatic compound PTFP (▲), para-substituted compounds (□), meta-substituted compounds (●) and ortho-substituted compounds (○). Note the shift in selectivity from JH esterase inhibition by the aliphatic series toward α-napthyl acetate esterase inhibition by the ortho-substituted aromatic compounds.*

standard assay conditions hydrolyze JH I faster than JH III (26–29), the fact that the optimum JH esterase inhibitors in two distinct series (Fig. 3) have MR values which are almost identical to those of the backbones of JH I and II provides further circumstantial evidence that the JH esterases are involved in JH regulation in the Lepidoptera.

Among the aliphatic sulfides, only the decyl sulfide **(D)** demonstrated significant inhibitory activity on trypsin. The activity on AChE and α-NA esterase appeared very similar through the aliphatic series, with the moderate activity of the cyclohexyl sulfide **(G)** on α-NA esterase and the selectivity demonstrated by benzyl sulfide **(H)** being of special note.

Within any one series of compounds, the inhibition of JH esterase activity (largely a single enzyme) and α-NA esterase activity (multiple enzymes) from the hemolymph *T. ni* were correlated (Fig. 4). In general, the aliphatic series of compounds (**A–F**, Table 1) were more selective for JH esterase, and the *ortho*-substituted phenyl compounds (**J, M, R, W,** and **Y**, Table 1) were more

selective for inhibiton of α-NA esterase activity. This observation is not surprising if one assumes that the compounds are acting as transition-state analogs since the aliphatic series and, to a lesser extent, the *para*-substituted phenyl series are steric mimics for the natural substrates.

In a previous manuscript the inhibitory activity on JH esterase of 29 trifluoromethylketones lacking the sulfide bond were examined. These compounds have I_{50}s roughly 50 times higher than similar compounds with the sulfide bond (Table 1). This enhanced activity of the sulfide series on JH esterase was not surprising, since sulfides are commonly used to mimic olefins in pharmaceutical chemistry, and the sulfide is in the correct position to mimic the 2,3-olefin of JH.

Comparison of the inhibitory potency on α-NA esterase and AChE of several pairs of compounds in Table 1 (with sulfide bonds) and Table 2 (without sulfide bonds) indicates that the former are more active. Reasonable comparisons include **B** vs **VI**, **D** vs **VII**, **E** vs **VIII**, and **I** vs **X**, **XI**, and **XII**. The latter three compounds illustrate that the distance from the phenyl ring to the carbonyl has little influence on inhibitory potency with the compounds examined. It is possible that the sulfur interacts with some functionality near the active site of these enzymes, but it is also possible that the sulfur stabilizes the form of the inhibitors which approach transition-state structures.

The slopes of the inhibition curves for the trifluoropropanone sulfides on JH esterase and α-NA esterase (Table 1) are much steeper than the slopes of the inhibition curves for the trifluoromethylketones lacking the sulfide bond (Table 2, Ref. (2)). This difference is nicely illustrated by the shallow slope of TFT in Fig. 2. The inhibition slopes of the trifluoropropanone sulfides are relatively independent of enzyme concentration (although dependent on preincubation time). In contrast, the I_{50}s and slopes of the TFT inhibition curves are

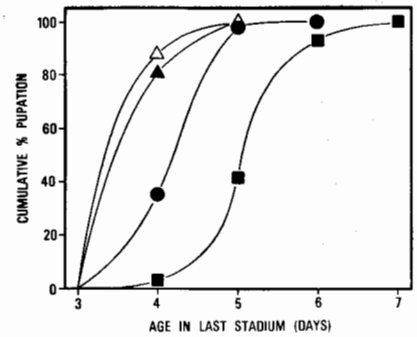


FIG. 5. *Cummulative delayed pupation in last stadium T. ni from topical application of 0.1 μmol EPPAT (■), OTFP (●) and TFT (△) at three times per day for the first 2 days of the last larval stadium. The control (▲) received 1 μl ethanol. Each treatment consisted of at least 40 larvae. The time needed for 50% of the larvae to pupate (T_{50}) was determined graphically as shown above for the 0.1 μmol dose. The graphs for the 0.2 μmol doses are not shown. Control (T_{50} = 3.5 days); EPPAT, 0.1 μmol (5.1); EPPAT, 0.2 μmol (4.2); OTFP, 0.1 μmol (4.1); OTFP, 0.2 μmol (4.4); TFT, 0.1 μmol (3.4); and TFT, 0.2 μmol (3.8).*

rather time independent. Although the I_{50}s change little with changes in enzyme concentration, the slopes increase with a decrease in enzyme concentration.

This difference is due to the detailed mechanism of inhibition to be discussed more extensively in later publications (Abdel-Aal, unpublished). The trifluoropropanone sulfides appear to be slow, tight-binding inhibitors while the trifluoromethylketones are classical competitive inhibitors with a higher on/off rate which leads to more shallow slopes for the latter compounds. From a practical standpoint these data indicate that the trifluoropropanone sulfides will be more useful tools for inhibiting JH esterase *in vitro* in experiments where high levels of inhibition are desired. Also, they will be better inhibitors than trifluoromethylketones of similar I_{50}s under *in vivo* conditions where the concentration of enzyme is high.

In Vivo Activity

As shown in Fig. 5, OTFP was effective in delaying the pupation of the larvae of *T. ni* when repeated doses of 0.1 μmol were

applied during the first 2 days of the last larval stadium. The effects were more dramatic when doses of 0.2 μmol/application were used, indicating a dose dependence. As expected, neither dose of TFT resulted in as great of a delay in pupation as OTFP, while the irreversible inhibitor EPPAT proved to be more potent than OTFP in delaying pupation at 0.1 μmol/application. No mortality was observed with repeated 1-μl applications of ethanol, TFT, or OTFP, while repeated applications of 2 μl ethanol, the TFT solution, or the OTFP solution (0.2 μmol/application, ca. 1000 mg/kg) resulted in 1, 6, and 4% mortality, respectively. In contrast, EPPAT gave 23 and 49% mortality (largely occurring at larval–pupal transformation) at the low and high doses, respectively.

The 48-hr mouse LD_{50} was greater than 750 mg/kg for OTFP, in that 33% of the mice died at this concentration in 48 hr. Additional mortality occurred within 2 weeks of the injection; 100% killed at 750 mg/kg, 50% at 500 mg/kg and 33% at 250 mg/kg. No mortality was observed in DMSO-treated control mice.

CONCLUSION

In an earlier study (2) TFT was observed to be an active competitive inhibitor of JH esterase, yet it failed to produce the *in vivo* effects expected of a JH esterase inhibitor (delayed pupation for example), as observed with the phosphorylating reagent EPPAT, even though the *in vitro* I_{50} of TFT is 10 times lower than that of EPPAT in *T. ni* (29, 30). This observation could be anticipated, since reversible inhibitors usually must be very powerful to elicit *in vivo* responses such as delayed pupation, and since previous studies showed that the majority of the JH esterase present in a larva must be inhibited for *in vivo* activity (30).

OTFP (B), which has an I_{50} almost 50 times lower than TFT does, demonstrates the expected *in vivo* effects in *T. ni* larvae. As shown in Fig. 5, application of the OTFP to prewandering larvae results in a significant delay in pupation. This effect has previously been demonstrated by the application of JH, juvenoids, or the JH esterase inhibitor EPPAT (2, 29–31). It is suspected that maintenance of a high JH titer by exogenous application of JH or its mimics results in a delay in the release of the neurohormone (prothoracicotrophic hormone) which initiates the pupation sequence. Thus, if JH esterase has a biological rule in the clearance of JH from early last-stadium larvae, its inhibition should result in a high JH titer and a subsequent delay in pupation.

Such evidence for the role of JH esterase in the clearance of JH has been generated in several species (2, 28–30, 32, 33). However, these studies do not prove unequivocally that JH esterase is crucial for the regulation of the JH titer. For instance, EPPAT is active against α-NA esterase from *T. ni* larvae and AChE as well. When comparing the I_{50} of OTFP on JH esterase versus α-NA esterase and electric eel AChE, OTFP was found to be 478 and 43000 times more active on JH esterase, and *in vivo* mortality from OTFP application was minimal. The *in vivo* inhibition of JH esterase and α-NA esterase were as could be anticipated from the I_{50}s (34). Both EPPAT and OTFP are known to inhibit other enzymes, and can be expected to inhibit still others with partially overlapping selectivities. Thus, the fact that a delay in pupation was observed with compounds in two structurally diverse classes, and that OTFP appears to have few side effects, provides further evidence for an *in vivo* role for JH esterase in regulating JH titers in lepidopterous larvae.

The low acute toxicity of OTFP to mice indicates that it is probably a reasonably safe probe for use by insect physiologists. However, the nature of the delayed effects is not understood, and the compound should be treated with caution. Other members of the substituted thio-1,1,1-trifluoro-2-propanone series and related compounds can be anticipated to be potent toxins and should be treated with caution.

Polarized ketones have now been shown to inhibit a wide variety of serine esterases and proteases from several diverse species ((1, 2, 4, 34), unpublished). As shown by this study, a sulfide bond β to the carbonyl can greatly increase the inhibitory potency of these compounds, as well as result in a straightforward, high-yield synthetic method. By varying the substituents about the sulfide linkage, it appears possible to devise potent, selective inhibitors for a wide variety of enzymes of practical interest.

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