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Synthesis and Bioassay of Isoprenoid 3-Alkylthio-1,1,1-trifluoro-2-propanones: Potent, Selective Inhibitors of Juvenile Hormone Esterase[1]

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Four 3-alkylthio-1,1,1-trifluoro-2-propanones with juvenile hormone-like side chains were prepared from citronellol and homogeraniol. These substrates were designed as possible transition-state analogs for the juvenile hormone (JH)-specific esterases present in insects. These four isoprenoid trifluoromethyl ketones were assayed *in vitro* with JH esterase and general esterases from larvae of the cabbage looper, *Trichoplusia ni* (Lepidoptera, Noctuidae), and with eel acetylcholinesterase and bovine chymotrypsin. JH esterase inhibition I_{50} values were in the nanomolar range for all four compounds, while the other esterases had I_{50}'s which were 10^3 to 10^5 higher. The high selectivity of these inhibitors is believed to be due to their similarity in size and functionality to natural JH III. Treatment of *T. ni* larvae *in vivo* with solutions of the most active analog, 3-[(E)-4,8-dimethyl-3,7-nonadienylthio]-1,1,1-trifluoro-2-propanone (DNTFP) causes a dose-dependent delay in pupation and a concurrent selective inhibition of JH esterase. These data support the hypothesis that the reduction in *in vivo* JH titer in larval *T. ni* is due, in part, to hydrolysis of the hormone by selective esterases. DNTFP appears to be competing with JH for the active site of JH esterase.

Juvenile hormones (JHs)[5] serve as morphogenetic and vitellogenic regulators in insects (1). The two key catabolic events in the reduction of JH titer in insects are epoxide hydrolysis and methyl ester hydrolysis, and in the Lepidoptera ester hydrolysis appears to predominate (2, 3). In contrast to endocrine regulation in vertebrates where changes in the biosynthetic rate play the dominant role, the activities of enzymes responsible for JH catabolism change dramatically during the development of several insect groups. Elucidation of the biological role of the JH esterases

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[3] Postdoctoral research fellow supported by National Service Award GM09223 from the National Institute of General Medical Sciences.

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[5] Abbreviations used: JH, juvenile hormone; JHE, JH esterases; THF, tetrahydrofuran; DNTFP, 3-[(E)-4,8-dimethyl-3,7-nonadienylthio]-1,1,1-trifluoro-2-propanone; α-NAE, α-naphthyl acetate esterase; AChE, acetylcholinesterase; α-ChT, α-chymotrypsin; TFT, 1,1,1-trifluorotetradecan-2-one; OTFP, 3-octylthio-1,1,1-trifluoro-2-propanone; EPPAT, O- ethyl-S-phenylphosphoramidothioate; ALO, after lights on.

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(JHE) from insect tissues (4-6) has been aided by a rapid JHE assay (7) and the availability of potent, selective JHE inhibitors (8-10) (Fig. 1). 1,1,1-Trifluorotetradecan-2-one (TFT) emerged as a potent, reversible apparent transition state analog inhibitor of larval JHE from *Trichoplusia ni in vitro* (8) but lacked efficacy *in vivo*. Substitution of sulfur for the C-4 methylene as in 3-octylthio-1,1,1-trifluoro-2-propanone (OTFP) increases the potency of these analogs, possibly by mimicking the electron density of the alkene bond of natural JHs (B. D. Hammock, Y. Abdel-Aal, C. M. Mullin, T. Hanzlik, and R. M. Roe, unpublished results). We now report the preparation and esterase inhibition of four 3-alkylthio-1,1,1-trifluoro-2-propanones (1-4) containing terpenoid side chains designed to mimic JH III (Fig. 2). We also report *in vitro* and *in vivo* data which demonstrate the specificity of compound 3, DNTFP, for JHE relative to other esterases.

EXPERIMENTAL PROCEDURES

General synthetic procedures. The synthetic schemes are summarized in Fig. 2. Infrared spectra were obtained on a Perkin-Elmer 727 instrument. ^{1}H NMR spectra were recorded at 80 MHz on a Varian HFT-80 spectrometer or at 300 MHz on an NT 300 instrument. ^{13}C NMR spectra were recorded at 20 MHz on a Varian CFT-20 or at 75 MHz on an NT 300 high-field spectrometer. All NMR samples were prepared in $CDCl_3$. Shifts are reported in ppm relative to δ (TMS) = 0 ppm.

Thin-layer chromatography was performed using MN Polygram Sil G/UV 254 (4 × 8 cm) TLC plates

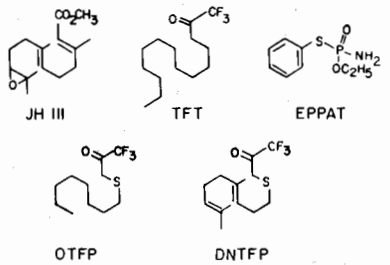


FIG. 1. Substrate (JH III, juvenile hormone) and inhibitors for JH esterase: OTFP, 3-octylthio-1,1,1-trifluoro-2-propanone; DNTFP, 3-[(E)-4,8-dimethyl-3,7-nonadienylthio]-1,1,1-trifluoro-2-propanone; TFT, 1,1,1-trifluorotetradecan-2-one; EPPAT, *O*-ethyl-*S*-phenylphosphoramidothioate.

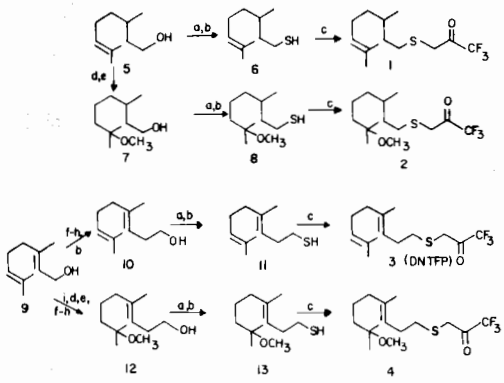


FIG. 2. Synthesis of trifluoromethyl ketones. Reagents: (a) DIAD, Ph_3P, $CH_3C(O)SH$; (b) $LiAlH_4$, THF; (c) $BrCH_2C(O)CF_3$, $NaHCO_3$, CH_2Cl_2; (d) $Hg(OAc)_2$, CH_3OH; (e) $NaBH_4$, C_2H_5OH; then ^-OH; (f) PBr_3, $(C_2H_5)_2O$; (g) NaCN, DMSO; (h) KOH, H_2O, CH_3OH; (i) Ac_2O, Py. See Experimental Procedures for details.

visualized with ethanolic-vanillin-H_2SO_4 reagent. Flash chromatography was performed on Merck Silica Gel G (400-230 mesh) under N_2 pressure. Tetrahydrofuran (THF) was distilled under N_2 from sodium benzophenone ketyl. All other solvents were Fisher HPLC grade unless otherwise stated.

3,7-Dimethyl-6-octene-1-thiol (6). Thiols were prepared from the corresponding alcohols via the *S*-acetyl derivatives using a Mitsunobu procedure described by Volante (11). Synthetic details are presented here for the citronellol (5) to citronellylthiol (6) conversion. Thus, 5.3 ml (0.027 mol) of di(isopropyl) azodicarboxylate (DIAD) was injected into the flask containing a solution of triphenylphosphine (6.7 g, 0.027 mol) in 100 ml of dry THF which was stirred efficiently at 0°C. A yellow precipitate formed at once and the suspension was stirred at 0° for 1 h. Then a solution of 2.3 ml (0.013 mol) of racemic citronellol (Aldrich Chemical Co.) and 2 ml (ca. 0.027 mol) thiolacetic acid in 50 ml THF was added dropwise over 10 min into the suspension. The mixture was stirred for 2 h (0-20°C), concentrated to give 6 g of residue, and kept at 4°C for 12 h. The liquid was decanted and the solid part was triturated with pure hexane. The solutions were combined and purified by flash chromatography (12) with 5% ethyl acetate in hexane. A crude product of 2.3 g (83%) of citronellyl thioacetate was obtained which was sufficiently pure for the subsequent hydrolysis. The only major contaminant was the corresponding thiol as determined by TLC (20% ethyl acetate-hexane): thiolester, R_f = 0.56; thiol, R_f = 0.67). Flash chromatography (2% ethyl acetate-hexane) gave a homogeneous product (>89% by GC) which was used for the spectra. IR (neat film), 2900, 1690, 1650, 1140 cm^{-1}; ^{1}H NMR, δ5.03 (t, J = 7 Hz, H-6),

2.50 (t, J = 6.5 Hz, H-1), 2.03 (s, acetyl CH_3); 1.94 (m, H-5); 1.65, 1.57 (s, s, H-8, H-9), 0.88 (d, J = 5.8 Hz, H-10).

The crude thiolester (2.3 g, 0.011 mol) was dissolved in 25 ml ether and added dropwise to a suspension of 0.8 g of $LiAlH_4$ (0.02 mol) in 20 ml ether under N_2. The mixture was stirred for 2.5 h at room temperature, and then 15 ml of 1 N HCl was added to destroy the excess $LiAlH_4$. The organic layer was separated and concentrated to give 1.3 g of crude product (69%). Evaporative distillation (80°C/0.05 mm Hg) gave 1.10 g of a colorless liquid (6) which was homogeneous by TLC (15% ethyl acetate–hexane, R_f = 0.58). IR (neat), 2920, 2580, 1650, 1450, 1380 cm^{-1}; ^1H NMR, δ5.02 (t, 7 Hz, H-6), 2.48 (t, H-1), 1.92 (m, H-5), 1.65, 1.55 (s, s, H-8, H-9), 0.88 (d, 5.5 Hz, H-10).

3-[(3RS)-3,7-dimethyl-6-octenylthio]-1,1,1-trifluoro-2-propanone (1). The alkylation conditions which minimized side products also required prolonged reaction times. Thus, 0.14 g of thiol 6 (0.81 mmol) and 0.30 g (3.6 mmol) of solid $NaHCO_3$ were added to a solution of 0.18 g of 3-bromo-1,1,1-trifluoro-2-propanone (0.95 mmol) (Columbia Organics) in 20 ml CH_2Cl_2. The mixture was stirred under N_2 at room temperature for 5 days and then poured into 50 ml H_2O. After extraction with ether and washing with brine, the solvent was evaporated to give 0.20 g of the crude trifluoromethyl ketone. Purification on a flash column with 10% ethyl acetate–hexane and then distillation gave 0.14 g (61%) of a colorless product which lacked the ^1H resonances (δ2.85, 3.75 + 3.83) attributed to the hydrated form (13). ^1H NMR, δ5.03 (t, 7 Hz, H-6), 3.44 (s, H-3′), 2.50 (t, 6.4 Hz, H-1), 1.90 (m, H-5), 1.62, 1.58 (s, s, H-8, 9), 0.87 (d, 6 Hz, H-10).

3-[(3RS)-3,7-dimethyl-7-methoxy-octylthio]-1,1,1-trifluoro-2-propanone (2). Citronellol (5) was converted to methoxy alcohol 7 by methoxymercuration–basic borohydride reduction (14). Thiolation of 5.2 g (0.028 mol) as described above gave 3.0 g (0.015 mmol, 54%) of chromatographed thiol 8. Sodium bicarbonate-catalyzed alkylation of 90 mg (0.45 mmol) of 8 with bromotrifluoroacetone as described above gave 100 mg (72%) of trifluoromethylketone 2 after chromatography (15% ethyl acetate–hexane) and distillation. ^1H NMR, δ3.45 (s, H-3′), 3.15 (s, OCH_3), 2.50 (t, 8 Hz, H-1), 1.15 (s, H-8, 9), 0.87 (d, 6 Hz, H-10).

3-[(E)-4,8-dimethyl-3,7-nonadienylthio]-1,1,1-trifluoro-2-propanone (3, DNTFP). Homogeraniol 10 was prepared from geraniol 9 by bromination, cyanide homologation, hydrolysis, and hydride reduction (15). Homogeraniol (1.50 g, 8.9 mmol) was converted to the corresponding thiol 11 (1.00 g, 61% chromatographed yield) as described above. Alkylation of 120 mg (0.65 mmol) of thiol 11 with excess bromotrifluoroacetone gave 110 mg (0.37 mmol) of chromatographed (10% ethyl acetate–hexane), distilled DNTFP 3 (56%). ^1H NMR, δ5.00 (m, H-3, 7), 3.40 (s, H-3′), 2.40 (t, H-1), 1.93 (s, H-11), 1.62, 1.55 (s, s, H-9, 10).

3-[(E)-4,8-dimethyl-8-methoxy-3-nonenylthio]-1,1,1-trifluoro-2-propanone (4). Geranyl acetate was converted to (E)-3,7-dimethyl-7-methoxyoct-2-en-1-ol by methoxymercuration–basic borohydride reduction followed by chromatographic removal of the dimethoxy material (14). Homologation of this material to (E)-4,8-dimethyl-8-methoxynon-3-en-1-ol (12) was accomplished as for geraniol (15). The alcohol 12 (1.00 g, 5.0 mmol) was converted to the corresponding thiol 13 (0.70 g, 3.2 mmol) in 64% yield after chromatography and distillation. Sodium bicarbonate-catalyzed alkylation of the thiol (0.090 g, 0.41 mmol) with excess bromotrifluoroacetone in CH_2Cl_2 gave 0.079 g of ketone 4 (62%) after chromatography (20% ethyl acetate–hexane) and evaporative distillation: ^1H NMR, δ5.00 (t, 6.5 Hz, H-3), 3.42 (s, H-3′), 3.10 (s, OCH_3), 2.3 (m, H-1), 1.45 (s, H-11), 1.13 (s, H-9, 10).

Insect rearing and hemolymph collection. Larvae of *Trichoplusia ni* (Hübner) were reared 20 per 240-ml cup at 27±2°C and LD 14:10 on diet No. 1 (6). Hemolymph from the first and second day of the fifth stadium (L5D1 to L5D2) was collected from clipped anal prolegs while hemolymph from L5D4 larvae was collected from clipped thoracic legs. Hemolymph was collected in a 6 × 50-mm culture tube at 4°C and centrifuged for 3 min at 1000*g*. The plasma was then diluted (v/v) with sodium phosphate buffer (0.08 M, pH 7.4, 0.01% phenylthiourea) and stored at −60°C until used for enzyme assays. Minimal loss of enzyme activity was observed upon freezing.

Enzyme assays. Plasma JHE activity was assayed by the method of Hammock and Sparks (7). The substrate, [10-^{3}H]JH III (NEN) was mixed with unlabeled JH III to obtain a final concentration of 5.0×10^{-6} M when 1 μl of substrate in ethanol was mixed with 100 μl of 1:500 diluted plasma. α-Naphthyl acetate esterase (α-NAE) activity was assayed according to the procedure of Sparks *et al.* (16). One milliliter of α-NAE in phosphate buffer (as above) was mixed with 100 μl of 1:10 diluted plasma to give a final substrate concentration of 2.3×10^{-4} M. Plasma dilutions and incubation times were chosen that resulted in a linear hydrolysis rate with time and which approached the maximum hydrolysis rate at 30°C.

Acetylcholinesterase (AChE) activity was assayed at 37°C (17). Twenty microliters of the substrate acetylthiocholine iodide (Sigma) in sodium phosphate buffer (0.05 M, pH 7.2) was added to 3 ml of $1:3 \times 10^8$ (w/v) diluted Type VI-S electric eel acetyl cholinesterase (Sigma) in the same buffer to give a final substrate concentration of 5.0×10^{-4} M. α-Chymotrypsin (α-ChT) activity was measured at 25°C by the method of Bender *et al.* (18). Fifty microliters of the substrate *p*-nitrophenyl trimethylacetate (Aldrich) in acetonitrile and 0.1 ml of bovine pancreas Type II α-Chymotrypsin (Sigma) in sodium acetate buffer (0.01 M, pH 4.6) was added to 2.95 ml of sodium phosphate buffer (0.01 M, pH 8.0) to obtain a final enzyme dilution

of 1:600 (w/v) and a substrate concentration of 5.8×10^{-5} M. Enzyme concentrations and incubation times were chosen that resulted in a linear hydrolysis rate with time.

Inhibitors were preincubated 10 min with enzyme prior to the addition of substrate. Preincubation temperature was the same as the enzyme assay temperature. All inhibitors were in ethanol with 1 μl per assay for JHE and α-NAE assays, 3 μl for AChE, and 30 μl for α-ChT. The I_{50} values were determined from final inhibitor concentrations of 1×10^{-4} to 1×10^{-10} using least-squares regression formulas. JHE activity was from L5D2 larvae and α-NAE activity from L5D4 larvae.

Substrate-inhibitor competition (Table II). Larval hemolymph of *T. ni* (L5D2, 1:500 diluted plasma) was incubated for 0.5 min at 30°C, with inhibitor (TFT or DNTFP) and then 15 min with JH III substrate (Inhibitor first). Plasma was also incubated at 30°C, 0.5 min with JH III and then inhibitor was added and the incubation continued for an additional 14.5 min (Substrate first). For control experiments, inhibitor was replaced with ethanol only.

In vivo assays. Larvae were treated on the dorsum of the thorax with 1 or 2 μl of ethanol (control) and with 1 or 2 μl, respectively, of 10^{-1} M TFT, OTFP, DNTFP, or EPPAT (*O*-ethyl-*S*-phenylphosphoramidothioate) in ethanol, three times per day at 4, 12, and 17 h ALO (after lights on) on L5D1 and D2. Time of pupation was checked daily at 4 h ALO, and emergence was checked 2 weeks after treatment. L5D1 larvae 4 h ALO were also treated but only once with 1 μl ethanol or with 1 μl of 10^{-1} M TFT, OTFP, DNTFP, or EPPAT in ethanol, and then were bled at 1, 8, 12, 23, 28, or 32 h after treatment. Hemolymph was pooled from five larvae, mixed, and frozen at −60°C until assayed for JHE and α-NAE activity. Freezing had essentially no effect on enzyme activity. The EPPAT was generously provided by T. R. Fukuto (UC Riverside).

RESULTS AND DISCUSSION

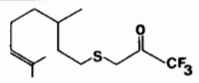
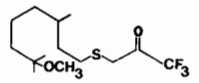
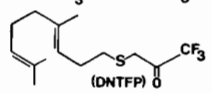
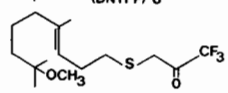
The reaction scheme (Fig. 2) was optimized using the readily available citronellol, which provided 12-atom-chain JH mimics 1 and 2. On the basis of structural congruence to JH III, we suspected the analogous 13-atom-chain compounds 3 and 4 to show higher inhibitory activity for JHE. Conversion of citronellol to the corresponding thiol 6 was accomplished in 69% yield using a modification of the Mitsunobu reaction (11). Thus, the thioacetate was prepared using diisopropylazodicarboxylate (DIAD), triphenylphosphine, and thiolacetic acid, and the free thiol was liberated by hydride reduction. Treatment of 6 with 3-bromo-1,1,1-trifluoro-2-propanone in the presence of a base gave the 3-alkylthio-1,1,1-trifluoro-2-propanone 1. Bases such as ethanolic sodium ethoxide (19) and triethylamine (20) in CH_2Cl_2 gave low yields of 1 and complex product mixtures, but stirring thiol (6) with solid $NaHCO_3$ in CH_2Cl_2 for 120 h gave clean conversion to 1 (^{1}H NMR, δ3.45, s, H-3), which was purified by flash chromatography followed by bulb-to-bulb distillation (80°C/0.1 mm, 61% yield from the thiol). Prior to distillation, a substantial proportion of the hydrated ketone was present, as seen in the ^{1}H NMR (δ2.85, s, H-3; 3.75 + 3.83, OH).

The second citronellol-derived compound 2 was prepared from (*R*)-3,7-dimethyl-7-methoxyoctan-1-ol (7), which had been obtained by methoxymercuration of citronellol. Conversion to thiol 8 proceeded in 54%, while alkylation with bromotrifluoroacetone ($NaHCO_3$ method) gave 2 in 72% distilled yield. Inhibitors 3 and 4 were prepared by homologation of geraniol (or its methoxy derivative) and then reaction as described above for conversion to the thiols and 3-alkylthio-1,1,1-trifluoro-2-propanones. The trifluoromethyl ketones 3 and 4 are close steric and functional mimics for JH III, and should optimize the specificity for JHE while retaining the desired low effective inhibitory concentration.

All four trifluoromethyl ketones are exceptionally powerful inhibitors of JH esterase with I_{50} values of 3 to 8×10^{-9} M (Table I) for JH III as substrate. Although JH II is the major JH for *T. ni*, JH III was selected as a substrate on the basis of availability, cost, and water solubility. Independent JHE assays with JH II and JH I by Y. Abdel-Aal (unpublished results) confirm that at physiological substrate concentrations (e.g., 10^{-9} M), the specificity of JHE for the three labeled JH analogs as measured by k_{cat}/K_m or V_{max}/K_m, is essentially unity. The homologated (13-atom chain) analogs 3 and 4 are slightly better than the citronellol-derived products, although the difference is less than originally anticipated. The I_{50} of DNTFP (analog 3) is approximately two orders of magnitude lower than that for the all-carbon TFT

TABLE I

INHIBITION OF JUVENILE HORMONE ESTERASE, α-NAPHTHYL ACETATE ESTERASE, ACETYLCHOLINESTERASE, AND α-CHYMOTRYPSIN ACTIVITY

	Compound	I_{50} (M)[a] JHE	α-NAE	AChE	α-ChT
1		4.5×10^{-9}	6.7×10^{-7}	2.7×10^{-6}	$>10^{-4}$
2		7.6×10^{-9}	6.5×10^{-7}	1.1×10^{-4}	$>10^{-4}$
3		3.1×10^{-9}	1.9×10^{-6}	$>10^{-4}$	$>10^{-4}$
4		3.2×10^{-9}	7.2×10^{-7}	6.2×10^{-5}	$>10^{-4}$

[a] I_{50} is defined as the concentration of compound required to inhibit 50% of the enzyme activity. Assays were performed at least in duplicate at each inhibitor concentration.

(Fig. 1; $I_{50} = 1 \times 10^{-7}$ M (8)) but about equal to the straight chain OTFP (Fig. 1; $I_{50} = 2.3 \times 10^{-9}$ M; B. D. Hammock, Y. Abdel-Aal, T. Hanzlik, C. M. Mullin, and R. M. Roe, unpublished results).

Within the OTFP series, the I_{50} is a function of the chain length with the lowest I_{50} corresponding to chain lengths approaching that of JH. The interaction of these inhibitors with JH esterase is strongly influenced by the 3-alkylthio-1,1,1-trifluoro-2-propanone moiety, with chain lengths approaching that of JH having reduced importance. Also, structural JH mimicry other than chain length has little effect on the I_{50} as demonstrated by the comparison of the I_{50} of DNTFP with that of OTFP. Selectivity is apparently also unaffected by structural JH mimicry. The I_{50} values for α-NAE activity (Table I) are two to three orders of magnitude higher than that for JHE activity, similar to the selectivity found for OTFP (B. D. Hammock, Y. Abdel-Aal, C. M. Mullin, T. Hanzlik, and R. M. Roe, unpublished results). For the most selective and most potent analog DNTFP (3), less than 50% inhibition of chymotrypsin and acetylcholinesterase activity is seen at concentrations 10^5 higher than the JHE I_{50}'s.

For both TFT and DNTFP, there was a reduction in percentage inhibition when substrate was added first (Table II). This observation suggests that the binding of JH III to JHE can block the binding of the inhibitors, either competitively or non-competitively. B. D. Hammock, Y. Abdel-

TABLE II

COMPETITION OF JH III AND INHIBITOR FOR JHE ACTIVE SITE

Inhibitor (M)	Percentage inhibition[a] Inhibitor first	Substrate first
TFT		
1×10^{-7}	57	31
5×10^{-7}	80	67
1×10^{-6}	86	79
DNTFP		
1×10^{-8}	31	9
5×10^{-8}	83	22
1×10^{-7}	94	43

[a] Measured for *T. ni* plasma (L5D2, 1:500 dilution) incubated at 30°C for 0.5 min with inhibitor first then 15 min with JH III, or at 30°C for 0.5 min with JH III first and then 14.5 min with inhibitor. For control, inhibitor was replaced by ethanol only.

Aal, C. M. Mullin, T. Hanzlik, and R. M. Roe (unpublished results) found that the most potent inhibitor in the 3-alkylthio-1,1,1-trifluoro-2-propanone series was OTFP with a molar refractivity of the alkyl chain (a correlate of steric bulk) very similar to that for JH III and JH II. If these inhibitors were acting noncompetitively, this would mean that the inhibitor-binding site would be separate but similar in structure to that for the active site of JHE, which appears unlikely. It is probable that DNTFP (3), which is similar in chain length to OTFP, is a competitive inhibitor of JHE. Y. Abdel-Aal, R. M. Roe, and B. D. Hammock (unpublished results) found that the 3-alkylthio-1,1,1-trifluoro-2-propanones are reversible inhibitors, but that classical Lineweaver–Burk analysis was not applicable because steady-state conditions were not evoked at the low inhibitor concentration needed to inhibit JHE. Inhibitor concentrations of 1×10^{-9} M was approaching the molar concentration of JHE in the assay used.

Finally, experiments with fifth-instar *T. ni* larvae demonstrated that DNTFP could retard pupation and suppress JHE activity *in vivo* in contrast to the trifluoromethyl ketones lacking the 4-thia substitution. The delay in pupation observed can be caused by JH application and would be the anticipated biological result if endogenous JH were not hydrolyzed. There was no difference in the time of pupation between control L5D1–L5D2 larvae treated with 1 and 2 μl of ethanol and larvae treated with 0.1 and 0.2 μmol of TFT (Fig. 3). For the same doses of OTFP, DNTFP, and EPPAT, pupation was delayed with respect to the controls in a dose-dependent manner (Fig. 3). The EPPAT-treated larvae showed statistically higher ($P < 0.05$) mortality rates which were dose dependent, 23% at 0.1 μmol and 49% at 0.2 μmol. Death usually occurred during the larval-pupal transformation. The control mortality at 2 μl of ethanol was 1%, and at 0.2 μmol for TFT was 6%, for OTFP was 4%, and for DNTFP was 0%.

EPPAT was used in a number of studies as an *in vitro* inhibitor of JHE (10, 21–23). Its *in vivo* effects have been documented

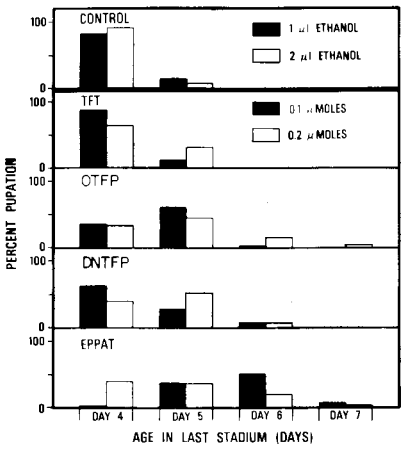


FIG. 3. Delayed pupation in last stadium *T. ni* from topical application of selected JHE inhibitors three times daily for L5D1 and L5D2. Each treatment represents at least 40 larvae. The average weights of L5D1 4 h ALO and 13 h ALO *T. ni* were 0.12 and 0.18 g/larva, respectively; for L5D2 4 h ALO and 13 h ALO, weights were 0.26 and 0.29 g/larva, respectively (16).

and utilized to study the role of JHE in JH regulation (9). *In vivo*, EPPAT is very effective against JHE and persistent for at least 32 h, but EPPAT inhibits α-NAE as effectively as JHE (Fig. 4). The nonselectivity of EPPAT might be the basis for mortality in the *in vivo* experiment.

Of the 3-alkylthio-1,1,1-trifluoro-2-propanones, OTFP and DNTFP were the most potent and most selective analogs in inhibiting JHE relative to α-NAE and in delaying pupation in treated larvae. TFT was found to be ineffective in producing delayed pupation (Fig. 3) and produced only a maximum of 40% inhibition for JHE and essentially no inhibition of α-NAE (Fig. 4). Both OTFP and DNTFP were more selective for JHE *in vivo* than EPPAT, and were as effective as EPPAT with respect to the maximum percentage inhibition obtained after treatment. EPPAT, however, was more persistent, less selective, and toxic. There was essentially no inhibition of α-NAE activity by OTFP, and DNTFP was intermediate to that of EPPAT and OTFP in this respect. Both JHE and α-NAE activity in the controls were increasing during the period, as seen in Fig. 4.

The use of TFT, OTFP, and DNTFP for

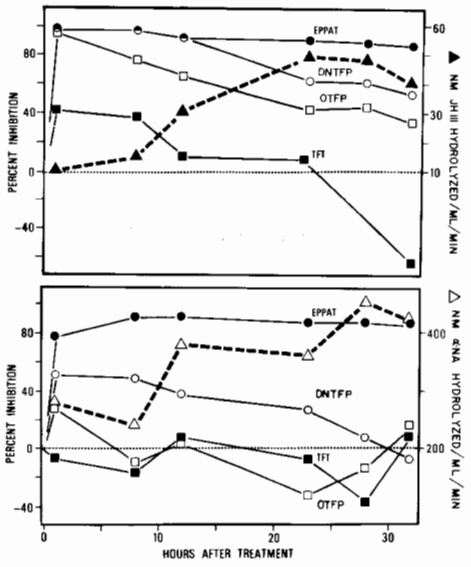


FIG. 4. *In vivo* inhibition of JHE in L5D1 to L5D2 larvae of *T. ni* treated with 0.1 μmole of inhibitor. Top panel shows inhibition of JHE activity; bottom panel shows inhibition of α-NAE activity. Each point represents the mean for at least 20 larvae. Normal JHE and α-NAE levels in control larvae are shown with triangles.

in vivo and *in vitro* inhibition of JHE in *T. ni* demonstrates their high activity and selectivity. Kinetic analyses for these compounds and other polarized ketones will be reported (Y. Abdel-Aal, R. M. Roe and B. D. Hammock) in due course. The application of these inhibitors to inhibition of JHE activity in other insects has considerable promise. The *in vivo* I_{50}'s of these compounds demonstrate them to be among the most potent of a class of novel inhibitors which may mimic the transition state of the target enzyme and its substrate. The *in vivo* data further support the hypothesis that ester hydrolysis by a specialized group of enzymes is important in JH regulation in at least some insects.

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