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

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Four 3-alkylthio-1,1,1-trifluoro-2-propanones with juvenile hormone-like side chains were prepared from citronellol and homog ergastone. These substrates were designed as possible transition-state analogs for the juvenile hormone (JH)-specific esterases present in insects. Three of these 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 cell acetylcholinesterase and bovine chymotrypsin. JH esterase inhibition IC50 values were in the nanomolar range for all four compounds, while the other esterases had IC50's which were 106-108 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, (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 JH titers in larval T. ni is due in part, to hydrolysis of the hormone by select esterases. DNTFP appears to be competing with JH for the active site of JH esterase.

Juvenile hormones (JHs) serve as morphogenetic and vitellogenic regulators in insects. (1) 4,8-dimethyl3,7-nonadienylthio-1,1,1-trifluoro-2-propanone, s-NAP, s-naphthyl acetate ester, AChE, acetylcholinesterase; s-C8T, s-cymatropine; CPT, 1,1,1-trifluorocyclopropane-2-one; OPPF, 3-alkylthio-1,1,1-trifluoroprop-2-ones; EPFAT, O-sulfonyl-S-

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Abreviations used: JH, juvenile hormone; JHE, JH esterases; T4PF, tetrahydrofurans; DNTFP, 3,8-di-
(JHE) from insect tissues (4-6) has been aided by a rapid JHE assay (7) and the availability of potent, selective JHE inhibitors (6-10) (Fig. 1). 1,1,1-Trifluoro-
trietrachloro-2-one (TFT) emerged as a po-
tect, reversible apparent transition state an-
alog inhibitor of larval JHE from Tri-
chophila sp in vitro (8) but lacked efficacy in vivo. Substitution of sulfur for the C-4 methylene as in 3-oxothithio-1,1,1-
trifluoro-2-propnones (OTFP) increases the potency of these analogs, possibly by mimicking the electron density of the alkene bond of natural JHs (B. D. Hammock, Y. Abeil-
Azi, C. M. Mullin, T. Haniluk, and R. M.
Roe, unpublished results). We now report the preparation and esterification of four 3-alkylthio-1,1,1-
trifluoro-2-propnones (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 com-
 pound 3, OTFP, for JHE relative to other esterases.

**EXPERIMENTAL PROCEDURES**

General synthetic procedures. The synthetic schemes are summarized in Fig. 3. Infrared spectra were ob-
tained on a Perkin-Elmer 737 instrument. 'H NMR
spectra were recorded at 80 MHz on a Varian HR-
60 spectrometer or at 200 MHz on an NT 300 instru-
ment. 13C NMR spectra were recorded at 20 MHz on a Varian CFT-36 or at 75 MHz on an NT 300 high-
field spectrometer. All NMR sample solutions were prepared in CDCl3. Shifts are reported in ppm relative to δ
TMS = 0 ppm.

Thin-layer chromatography was performed using MN Polygram Sil G/UV 244 (4 × 8 cm) TLC plates with

![FIG. 2. Synthesis of trifluoroethyl ketones. Re-
agents: (a) DiAD, Ph3P, CH2(OH)CH2OH; (b) LiAlH4, THF; (c) HCl/CH2Cl2, CH2OH; (d) NaH, CH2Cl2, then Ti CI (f) F2CC1,
(G3,5-0lig, (g) NaCN, DMF, H2O, CH2OH; (h) AlOH. Py. See Experimental Procedures for details.)](image)

**FIG. 1. Biosynthesis of JH III, juvenile hormone and inhibitors for JH esterase. OTFP, 3-oxothithio-1,1,1-
trifluoro-2-propnones; DFP, 3-[(R)-3-methyl-
3,3-dimethyl-2,2-dichlorophenyl]-1,1,1-
trifluoro-2-propenone; EPTP, 3-[4-ethyl-4-
phenylphosphinomethyl]benzoic acid.**
2.50 (3, J = 6.5 Hz, H-1), 2.08 (9, s, octet (7)), 1.94 (m, H-2), 1.37 (m, H-3), 0.98 (6, J = 5.5 Hz, H-10).

The crude thioester (2.8 g, 0.01 m mol) was dissolved in 25 ml ether and added dropwise to a suspension of 0.8 g of LiAlH4 (0.025 m) in 20 ml ether under N2. The mixture was stirred for 2.5 h at room temperature and then filtered and concentrated to give 1.3 g of crude product (69%).

Evaporative distillation (80°C/0.5 mm Hg) gave 1.10 g of a colorless liquid (6) which was homogeneous by TLC (10% ethyl acetate-hexane, Rf 0.38). (n) ment, 2280, 2290, 1640, 1480 cm−1. 1H NMR: 0.027, 2.4 (1 H, H-2), 2.48 (1 H, H-3), 1.53 (9 H, H-10), 0.86 (6, H, H-9). 13C NMR: 114.0, 115.5, 115.8 (C-4), 60.4 (C-6), 0.00 (C-5).

1.02 (0.7, d, 6.7Hz, CH3-O), 1.02 (0.7, d, 6.7Hz, CH3-O).

The allylation conditions which min-
imized side products also required prolonged reaction times. Thus, 0.14 g of thio 0.01 mmol) and 0.30 g (3.6 mmol) of NaI were added to a solution of 0.33 g of 3-bromo-1,1-dimethyl-2-propenyl (0.66 mmol) (Columbia (Organics) in 20 ml CH2Cl2. The mixture was stirred under N2 at room temperature for 5 days and then poured into 50 ml H2O. After extraction with ether, wash with brine, the solvent was evaporated to give 0.20 g of the crude trimethylsilylated ketone. Purification on a flash column with 10% ethyl acetate-hexane and then distillation gave 0.14 g (40%) of a colorless product which had the δ resonances (306.5, 3.73 at 8.18) attributed to the hydrated form (15). 1H NMR: 4.0 (2 H, H-1), 3.34 (2 H, H-2), 0.60 (3 H, H-9). 13C NMR: 40.2, 52.8, 14.0 (C-5).

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2.45 (4, H, H-4), 2.10 (1 H, H-2), 1.25 (9 H, H-10), 0.91 (6, H, H-9). 13C NMR: 114.0, 115.5, 115.8 (C-4), 60.4 (C-6), 0.00 (C-5).

2.45 (4, H, H-4), 2.10 (1 H, H-2), 1.25 (9 H, H-10), 0.91 (6, H, H-9). 13C NMR: 114.0, 115.5, 115.8 (C-4), 60.4 (C-6), 0.00 (C-5).

2.45 (4, H, H-4), 2.10 (1 H, H-2), 1.25 (9 H, H-10), 0.91 (6, H, H-9). 13C NMR: 114.0, 115.5, 115.8 (C-4), 60.4 (C-6), 0.00 (C-5). 13C NMR: 114.0, 115.5, 115.8 (C-4), 60.4 (C-6), 0.00 (C-5).
of 1.000 (v/v) and a substrate concentration of 5.8 × 10⁻³ 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 tempera- ture. 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-CH. The I₅₀ values were determined in a final inhibitor concentrations of 1 × 10⁻⁶ to 1 × 10⁻⁸ using least-squares regression formulas. JHE activity was from LSU1 larvae and α-NAE activity from LSU4 larvae.

Substrate-inhibitor competition (Table II). Larval hemolymph of T. ni (LSU1, 1.000 diluted plasma) was incubated for 0.5 min at 30°C, with inhibitor (TPT or DNTFF) 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 TPT, (2,4-DNTFF, or EFPTA (C₁₈-C₂₀-phosphophthalamide- dichloride) in ethanol, three times per day at 4, 12, and 17 h ALO (after lights on) on LSU1 and LSU. Time of pupation was checked daily at 4 h ALO, and emer- gence was checked 2 weeks after treatment. LSU1 and 4 ALO larvae were also treated hot only once with 1 μl ethanol or with 1 μl of 10⁻¹ M TPT, (2,4-DNTFF, or EFPTA in ethanol, and then fed at 1, 8, 12, 21, 24, or 24 h after incubation. Hemolymph was pooled from five larvae, mixed, and frozen at −60°C until assayed for JHE and α-NAE activity. Preincubation essentially had no effect on enzyme activity. The EFPTA was generously provided by T. R. Fukuto (UC River- side).

RESULTS AND DISCUSSION

The reaction scheme (Fig 2) was opti- mized using the readily available citro- nellol, 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 corre- sponding thiol 6 was accomplished in 69% yield using a modification of the Mitsunobu reaction (11). Thus, the thioacetate was prepared using disisopropylidicarboxy- llate (DIAD), triphenylphosphine, and thiolic acid, and the free thiol was lib- erated by hydride reduction. Treatment of 6 with 3-bromo-1,1,1-trifluoro-2-propanone 1. Bases such as ethanolic sodium ethoxide (19) and triethyamine (20) in CH₂Cl₂ gave low yields of 1 and complex product mixtures, but stirring thiol 6 with solid NaHCO₃ in CH₂Cl₂ for 120 h gave clean conversion to 1 (1H NMR, 83.45, s, H-3), which was purified by flash chromatography following by bulb-to-bulb distillation (10/0°C, 0.1% yield, 61% yield from the thiol). Prior to distil- lation, a substantial proportion of the hy- drated ketone was present, as seen in the H NMR (62.85, s, H-3, 3.78 and 3.83, OH).

The second citronellol-derived compound 2 was prepared from (R)-3,7-dimethyl-7- methoxyan-1-ol (7), which had been ob- tained by methoxycarbonylation of citro- nellol. Conversion to thiol 8 proceeded in 54%, while alkylation with bromoethio- roacetone (NaHCO₃ method) gave 2 in 72% distilled yield. Inhibitors 3 and 4 were pre- pared by homologation of geraniol (or its methoxy derivative) and then reaction as described above conversion to the thiol and 3-alkylthio-1,1,1-trifluoro-2-propanone. The trifluoromethyl ketones 3 and 4 are close stereoisomer and functional mimics for JH III, and should optimize the speci- ficity for JHE while retaining the desired low effective inhibitory concentration. All four trifluoromethyl ketones are ex- ceptionally powerful inhibitors of JHE ex- terase with I₅₀ values of 3 to 8 × 10⁻⁷ 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. In- dependent JHE assays with JH II and JH I by Y. Abdel-Aal (unpublished results) confirm that at physiological substrate concentrations (e.g., 10⁻⁵ M), the specificity for JHE for the three labeled JH analogs as measured by Kᵢ₅₀/Kₐ, or V₅₀/Kₐ, in es- sentially unity. The homologated (13-atom chain) analogs 3 and 4 are slightly better than the citronellol-derived products, al- though the difference is less than originally anticipated. The I₅₀ of DNTFF (analogue 3) is approximately two orders of magnitude lower than that for the all-carbon TPT
TABLE I
Inhibition of Juvenile Hormone Esterase, α-Naphthyl Acetate Esterase, Acetylcholinesterase, and α-Chymotrypsin Activity

<table>
<thead>
<tr>
<th>Compound</th>
<th>JHE</th>
<th>α-NAE</th>
<th>AChE</th>
<th>α-Cat</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.5 x 10⁻⁸</td>
<td>6.7 x 10⁻⁸</td>
<td>2.7 x 10⁻⁸</td>
<td>&gt;10⁻⁴</td>
</tr>
<tr>
<td>2</td>
<td>7.6 x 10⁻⁹</td>
<td>6.5 x 10⁻⁷</td>
<td>1.1 x 10⁻⁸</td>
<td>&gt;10⁻⁴</td>
</tr>
<tr>
<td>3</td>
<td>3.1 x 10⁻⁹</td>
<td>1.9 x 10⁻⁶</td>
<td>&gt;10⁻⁹</td>
<td>&gt;10⁻⁹</td>
</tr>
<tr>
<td>4</td>
<td>32 x 10⁻⁹</td>
<td>7.2 x 10⁻⁸</td>
<td>6.2 x 10⁻⁹</td>
<td>&gt;10⁻⁹</td>
</tr>
</tbody>
</table>

*I₀* 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: *I₀* = 1 x 10⁻⁵ M (86)* but about equal to the straight chain OTPF (Fig. 1; *I₀* = 2.3 x 10⁻⁹ M; R. D. Hammock, Y. Abdel-Aal, T. Hanlik, C. M. Mullin, and R. M. Roe, unpublished results). Within the OTPF series, the *I₀* is a function of the chain length with the lowest *I₀* 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-propanoic moiety, with chain lengths approaching that of JH having reduced importance. Also, structural JH mimery other than chain length has little effect on the *I₀* as demonstrated by the comparison of the *I₀* of DNTFP with that of OTPF. Selectivity is apparently also unaffected by structural JH mimery. The *I₀* 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 OTPF (B. D. Hammock, Y. Abdel-Aal, C. M. Mullin, T. Hanlik, 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 10⁹ higher than the JHE *I₀*.

For both TPT 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-Aal, T. Hanlik, C. M. Mullin, and R. M. Roe, unpublished results.

TABLE II
Comparison of JH III and Inhibitor for JHE Active Site

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Percentage inhibition*</th>
</tr>
</thead>
<tbody>
<tr>
<td>(µM)</td>
<td>Substrate first</td>
</tr>
<tr>
<td>TPT</td>
<td>1 x 10⁻⁵</td>
</tr>
<tr>
<td></td>
<td>5 x 10⁻⁶</td>
</tr>
<tr>
<td></td>
<td>1 x 10⁻⁴</td>
</tr>
<tr>
<td>DNTFP</td>
<td>1 x 10⁻⁴</td>
</tr>
<tr>
<td></td>
<td>5 x 10⁻⁴</td>
</tr>
<tr>
<td></td>
<td>1 x 10⁻⁳</td>
</tr>
</tbody>
</table>

*Measured for T. s. plasma (1500, 1500 dieth) 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. Mallin, T. Hamlik, and R. M. Roe (unpublished results) found that the most potent inhibitor in the 3-alkibio-1,1,1-trifluoro-2-propanone series was OTTP with a molar reactivity of 19. A 3-alkyl chain (a correlate of steric bulk) very similar to that for JHE 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 OTTP, is a competitive inhibitor of JHE. Y. Abdel-Aal, R. M. Roe, and B. D. Hammock (unpublished results) found that the 3-alkibio-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 x 10^{-8} 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 trifluormethy 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 Ld1/Ld2 larvae treated with 1 and 2 µl of ethanol and larvae treated with 0.1 and 0.2 µmol of TFP (Fig. 3). For the same doses of OTFP, DNTFP, and EPAT, pupation was delayed with respect to the controls in a dose-dependent manner (Fig. 3). The EPAT-treated larvae showed statistically higher (P < 0.05) mortality rates which were dose dependent, 23% at 0.1 µmol and 62% at 0.2 µmol. Death usually occurred during the larval-pupal transformation. The control mortality at 2 µl of ethanol was 13% and at 0.2 µmol was 6%. OTFP was 4%, and for DNTFP was 0%.

EPPAT was used in a number of studies as an in vivo inhibitor of JHE (10, 21–23). Its in vivo effects have been documented 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 EPAT inhibits a-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-alkibio-1,1,1-trifluoro-2-propanones, OTFP and DNTFP were the most potent and most selective analogues in inhibiting JHE relative to a-NAE and in delaying pupation in treated larvae. TFP 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 a-NAE (Fig. 4). Both OTFP and DNTFP were more selective for JHE in vivo than EPAT, and were as effective as EPPAT with respect to the maximum percentage inhibition obtained after treatment. EPAT, however, was more persistent, less selective, and toxic. There was essentially no inhibition of a-NAE activity by OTFP and DNTFP was intermediate to that of EPPAT and OTFP in this respect. Both JHE and a-NAE activity in the controls were increasing during the period, as seen in Fig. 4.

The use of TFP, OTFP, and DNTFP for
Fig. 4. In vivo inhibition of JHE in L1 and L2E larvae of T. ni treated with 0.1 μM 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 15 larvae. Normal JHE and α-NAE levels in control larvae are shown with triangles.

The 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. Arab-Aziz, R. M. Boe 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 fraction 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.

References