NEW POTENTIAL INHIBITORS OF PHEROMONAL ATTRACTION IN THE ORIENTAL FRUIT MOTH, *Cydia molesta*

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Received March 25, 1998
Accepted May 8, 1998

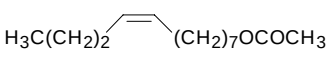
New analogues of (*Z*)-dodec-8-en-1-yl acetate (*Z*8-12:OAc, **1**), the main sex pheromone component of the Oriental fruit moth, *Cydia molesta*, were designed by formally transferring the terminal propyl group from the C-9 to the C-7 position to form vinyl-branched (**2**, **3**) or, after isomerization, ethylidene-branched (**4**) structures and by replacing the –CH=CH– grouping by the –S–CH_2– moiety (**5**, **6**). Their biological activities were studied both electrophysiologically and behaviourally (laboratory mating and wind tunnel experiments). All the structural modifications resulted in analogues whose electroantennographic activities were lower than that of **1** following the order **1** >> **6** ≈ **5** >> **2** ≈ **4** ≈ **3**. The single sensillum recording activities indicated that all the analogues stimulate the same *Z*8-12:OAc receptor neurone. In behavioural experiments, the analogues were generally found to reduce the ability of males to find a pheromone source, however, to different degrees. The highest inhibitory effect (90%) was observed for the thia analogues **5** and **6**. The results support the view that the inhibitory properties of the analogues should not be entirely associated with their pheromone-mimicking capabilities.

Key words: Pheromones; Sex attractants; Sex pheromone analogues; (*Z*)-Dodec-8-en-1-yl acetate; Oriental fruit moth; *Cydia molesta*; Electrophysiology.

Modifications of sex pheromone molecules have attracted considerable attention of chemists. The most common strategies which resulted in the synthesis of dozens of pheromone analogues[1,2] were based on replacement of different parts of pheromonal molecules with isosteric or reactive groups. While modifications of the acetate functionality predominated[3–8], modifications of carbon–carbon double bond(s) in pheromone structure were quite infrequent.

Perhaps the most unusual modifications of pheromone double bond(s), leading to significant biological consequences, were those based on formal moving the terminal alkyl chain to the allylic position of the double bond. Such "vinyl-branched" analogues were found[9] to inhibit effectively the male attraction to virgin females or to synthetic lures in the false codling moth, *Cryptophlebia leucotreta*. This finding initiated further

investigations of related-type analogues[10,11]. In contrast to Burger *et al*.[9], Mayer and Doolittle[11] observed that in the cabbage looper, *Trichoplusia ni*, the vinyl-branched analogues synergize the male behavioural response to the main pheromone component. Similar contrasting behavioural effects have been described for analogues based on a replacement of the –CH=CH– grouping by the $–CH_2–S–$ moiety[12,13]. The heterogeneous biological effects described above might be, at least in part, assignable to different kinds of insects investigated and to different bioassay methods used. However, the exact reasons why the same structural modification of the pheromone skeleton may result in completely opposite biological effects, remain unclear.



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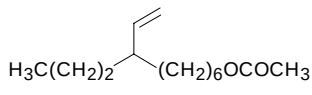

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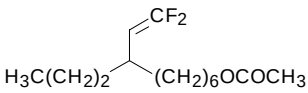

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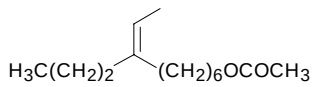

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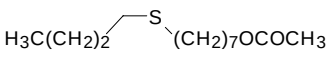

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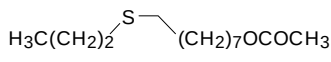

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In order to broaden our knowledge about the relation between the structure and activity of vinyl-branched and thia pheromone analogues, we tested both these analogue types on one model species, *viz*. the Oriental fruit moth (OFM) *Cydia molesta* (Lepidoptera, Tortricidae, Olethreutinae) using identical electrophysiological and behavioural bioassays. Thus in this paper, the biological activities of branched (**2–4**) and thia (**5**, **6**) analogues which modify the (*Z*)-8-double bond of (*Z*)-dodec-8-en-1-yl acetate (*Z*8-12:OAc, **1**), the main pheromonal component of the OFM female sex pheromone, are reported.

EXPERIMENTAL

Chemical Synthesis

NMR spectra were determined in $CDCl_3$ solutions on a Varian UNITY-500 spectrometer operating at 499.5 MHz for ^1H and at 125 MHz for ^{13}C NMR, respectively. Chemical shifts are expressed in δ (ppm) scale relative to TMS for ^1H and relative to $CDCl_3$ signal (77.00 ppm) for ^{13}C NMR, respectively. IR spectra (wavenumbers in cm^{-1}) were recorded on a Bruker IFS 88 FT-IR spectrometer in CCl_4 solutions while electron impact (70 eV) mass spectra were obtained on ZAB-EQ (VG, England) instrument. GC analyses were performed on a Hewlett–Packard HP 6890 chromatograph equipped

with a FID detector and a 30 m capillary column (i.d. 0.3 mm, HP5–5% cross-linked methyl(phenyl)-silicone). Preparative medium-pressure liquid chromatography (PMPLC) separations were made on Merck 60 silica gel (0.040–0.063 mm) using a Büchi B-680 Prep LC System with stepwise gradient of ethyl acetate in light petroleum.

All chemical reactions were run in oven dried glassware under inert atmosphere of argon or nitrogen. Tetrahydrofuran (THF) and ether were distilled from benzophenone ketyl, *N*,*N*-dimethylformamide (DMF) from P_2O_5. All other chemicals were used as purchased. The syntheses of analogues **3**, **5** and **6** are outlined in Schemes 1 and 2, respectively, while the analogues **2** and **4** have been described previously[10].

8-(*tert*-Butyldimethylsilyloxy)-2-propyl Octan-1-ol (**7**)

Butyllithium (1.6 M solution in hexane; 68.8 ml, 0.11 mol) was added to a stirred solution of dry diisopropylamine (10.6 g, 0.11 mol) in anhydrous THF (70 ml) at 0 °C. Pentanoic acid (5.1 g, 0.05 mol) was then added to the mixture and the temperature was kept below 0 °C. After 15 min hexamethylphosphorictriamide (HMPA; 19.8 ml, 0.11 mol) was added to the solution and the mixture was stirred at 5 °C for 15 min (ref.[14]). Finally, 1-bromo-8-(*tert*-butyldimethylsilyloxy)hexane (14.7 g, 0.05 mol) was added at 0 °C. After 4 h stirring at room temperature, the reaction was quenched with 10% HCl (200 ml). The organic layer was washed with brine (3 × 100 ml), dried over K_2CO_3 and evaporated. The residue (red oil, 14.3 g) was dissolved in anhydrous ether (20 ml) and added to a suspension of $LiAlH_4$ (3.8 g, 0.10 mol) in ether (100 ml). After refluxing for 3 h and subsequent cooling, the reaction mixture was decomposed with aqueous 10% NaOH (50 ml) and extracted with ether (3 × 100 ml). The combined extracts were washed with water and dried over K_2CO_3. Removal of the solvent *in vacuo* and purification of the residue by PMPLC gave 10.7 g (71%) of alcohol **7**. ^{1}H NMR spectrum: 0.08 s, 6 H (CH_3); 0.85 s, 9 H (CH_3); 0.86 t, 3 H, J = 7.2 (CH_3); 1.17–1.32 m, 12 H (CH_2); 1.43 m, 1 H (CH); 1.46 m, 2 H (CH_2); 3.49 d, 2 H, J = 5.5 (CH_2); 3.55 t, 2 H, J = 6.7 (CH_2). ^{13}C NMR spectrum: –5.31 (2 × CH_3), 14.42, 18.34, 19.99, 25.75, 25.95 (3 × CH_3), 26.82, 29.81, 30.85, 32.85, 33.23, 40.27, 63.29, 65.69. For $C_{17}H_{38}O_2Si$ (302.6) calculated: 67.48% C, 12.66% H; found: 67.67% C, 12.81% H.

8-(*tert*-Butyldimethylsilyloxy)-2-propyl Octanal (**8**)

A dichloromethane (3 ml) solution of alcohol **7** (1.0 g, 3.3 mmol) was oxidized with pyridinium chlorochromate[15] (1.07 g, 5.0 mmol) and sodium acetate (90 mg, 1.1 mmol) suspension in dichloromethane (10 ml). A usual work up and PMPLC afforded 0.9 g (91%) of the aldehyde **8**. ^{1}H NMR spectrum: 0.05 s, 6 H (CH_3); 0.89 s, 9 H (CH_3); 0.91 t, 3 H, J = 7.3 (CH_3); 1.26–1.66 m, 15 H (CH_2 and CH); 3.59 t, 2 H, J = 6.6 (CH_2); 9.55 d, 1 H, J = 3.2 (CHO). ^{13}C NMR spectrum: –5.29 (2 × CH_3), 13.97, 18.36, 20.27, 25.61, 25.96 (3 × CH_3), 27.05, 31.05, 32.11, 32.75, 34.33, 51.75, 63.19, 205.69. For $C_{17}H_{36}O_2Si$ (300.6) calculated: 67.94% C, 12.07% H; found: 68.11% C, 12.25% H.

9-(*tert*-Butyldimethylsilyloxy)-1,1-difluoro-3-propylnon-1-ene (**9**)

Tris(dimethylamino)phosphine (HMPT; 0.98 g, 6.0 mmol) in anhydrous DMF (5 ml) was added dropwise to a mixture of **8** (0.90 g, 3.0 mmol) and dibromodifluoromethane (1.33 g, 6.0 mmol) in the same solvent (5 ml) at 0 °C over a period of 0.5 h under argon[16]. The mixture was warmed to room temperature, stirred for additional 0.5 h, and then zinc dust (0.39 g, 6.0 mmol) was added in one portion. The mixture was stirred at 110 °C for 1 h, then poured into ice-water and extracted with ether. The combined organic phases were washed with water, dried over K_2CO_3, filtered and evaporated to yield an oily residue (0.5 g) which was purified by PMPLC. The chromatography gave 0.38 g

(38%) of **9**. ^{1}H NMR spectrum: 0.05 s, 6 H (CH_3); 0.88 t, 3 H, J = 7.1 (CH_3); 0.89 s, 9 H (CH_3); 1.13–1.54 m, 14 H (CH_2); 2.18 m, 1 H (CH); 3.59 t, 2 H, J = 6.6 (CH_2); 3.86 ddd, 1 H, J = 3.1, 10.5, 25.6 (CH=CF_2). ^{13}C NMR spectrum: –5.28 (2 × CH_3), 13.99, 18.38, 20.27, 25.75, 25.98 (3 × CH_3), 27.12, 29.39, 32.83, 33.50 (J(C,F) = 3.9), 35.66, 37.95, 63.27, 82.47 (J(C,F) = 19.5, 19.5), 156.61 (J(C,F) = 285.4, 285.4). For $C_{18}H_{36}F_2OSi$ (334.6) calculated: 64.62% C, 10.85% H, 11.36% F; found: 64.61% C, 10.65% H, 11.41% F.

9,9-Difluoro-7-propylnon-8-en-1-yl Acetate (**3**)

To a tetrabutylammonium fluoride (1 M solution in THF; 3.0 ml) silyl ether **9** (334 mg, 1.0 mmol) was added and the mixture was stirred for 24 h. The solvent was removed *in vacuo* and the residue was acetylated (acetic anhydride, pyridine, DMAP) in the usual manner. Subsequent purification by PMPLC afforded 149 mg (59%) of acetate **3**. ^{1}H NMR spectrum: 0.88 t, 3 H, J = 7.0 (CH_3); 1.13–1.42 m, 12 H (CH_2); 1.59–1.61 m, 2 H (CH_2); 2.05 s, 3 H, (CH_3); 2.18 m, 1 H (CH); 3.86 ddd, 1 H, J = 3.2, 10.5, 25.6 (CH=CF_2); 4.05 t, 2 H, J = 6.6 (CH_2). ^{13}C NMR spectrum: 13.97, 20.25, 21.00, 25.85, 27.03, 28.55, 29.19, 33.46 (J(C,F) = 3.9), 35.60, 37.93, 64.57, 82.40 (J(C,F) = 2 × 19.5), 156.61 (J(C,F) = 2 × 285), 171.24. For $C_{14}H_{24}F_2O_2$ (262.3) calculated: 64.10% C, 9.22% H, 14.48% F; found: 64.33% C, 9.41% H, 14.29% F.

7-(Butylsulfanyl)heptyl Acetate (**5**)

A solution of 7-bromoheptanol (100 mg, 0.51 mmol) and sodium butanethiolate (87 mg, 0.769 mmol) in dry DMF (2 ml) was stirred at 80 °C for 2 h. The reaction mixture was cooled to 0 °C, pyridine (400 μl) and acetic anhydride (400 μl) were successively added and the mixture was stirred at room temperature for 12 h. After quenching with ice-cold water and extraction with hexane, the combined organic phases were dried ($MgSO_4$), evaporated and the residue (105 mg) purified on silica gel to afford 50 mg (40%) of acetate **5**. ^{1}H NMR spectrum: 0.99 t, 3 H, J = 7.4, 7.4 (CH_3); 1.60–1.20 m, 14 H (CH_2); 2.05 s, 3 H (CH_3); 2.46 t, 2 H, J = 7.2, 7.6 (CH_2); 2.50 t, 2 H, J = 7.3, 7.3 (CH_2); 4.05 t, 2 H, J = 6.7, 6.8 (CH_2). IR spectrum: 1 741 (C=O); 1 239, 1 039 (C–O). Mass spectrum, *m/z* (%): 246 (M, 40), 203 (M – 43, 7), 143 (M – 60 – 43, 100), 84 (99), 69 (73), 61 (40), 55 (71), 43 (89).

7-(Propylsulfanyl)octyl Acetate (**6**)

Acetate **6** (64.1 mg; 54% yield) was prepared from 8-bromooctanol (100 mg, 0.478 mmol) and sodium propanethiolate (74 mg, 0.717 mmol) following the above described procedure. ^{1}H NMR spectrum: 0.92 t, 3 H, J = 7.4, 7.4 (CH_3); 1.6–1.2 m, 14 H (CH_2); 2.05 s, 3 H (CH_3); 2.51 t, 4 H, J = 7.0, 7.0 (CH_2); 4.05 t, 2 H, J = 6.7, 6.8 (CH_2). IR spectrum: 1 741 (C=O); 1 365 (CH_3O); 1 240, 1 039 (C–O). Mass spectrum, *m/z* (%): 246 (M, 54), 203 (M – 43, 7), 129 (M – 60 – 57, 100), 96 (50), 61 (68), 55 (79), 43 (54).

Determination of Vapour Pressures

The vapour pressures for compounds **1–6** were determined by the standard GC method[17]. The relative (to **1**) values were as follows: 1.00 (**1**), 1.72 (**2**), 1.83 (**3**), 1.18 (**4**), 0.098 (**5**) and 0.089 (**6**). To correct stimuli doses for volatility, the amounts of compounds (w_t) applied on filter paper discs were divided by the corresponding relative vapour pressure value.

Force Field Conformation Energy Calculations

Minimum energy calculations were performed for the *Z*- and *E*-isomers of hex-3-ene, (*Z*)-oct-4-ene, 3-methylhex-1-ene, and ethyl propyl sulfide. These models represented those parts of the molecule that were altered. Default values for bond angles and bond distances were taken from Van Hemelrijk *et al.*[18]. The energy of the compounds was minimized by MM+ software (HyperChem™, release 2, 1991, Autodesk Inc., U.S.A., using Polak–Ribiere conjugate gradient at 0.0042 kJ mol^{-1} termination conditions). Where appropriate, symmetry rules were used to find degenerated conformers. After the calculation of energy minimum, every generated conformer was treated by HyperChem's molecular dynamic procedure (T = 300 K, time 1 ps, step 0.001 ps and path relaxation 0.1 ps) and again minimized.

Insects

Oriental fruit moths, *Cydia molesta*, originated from a laboratory colony reared on a semiartificial diet under a 16 : 8 light : dark regimen at 20–25 °C. Pupae were sexed and males were kept separately from females under the same light and temperature conditions. Newly emerging adults were collected daily and provided with water and sugar solution on cotton wool. Males used were 2–3 days old for EAG experiments and 3–4 days old for behavioural observations.

Electrophysiological Experiments

a) *Electroantennography (EAG).* Ag/AgCl microelectrodes were used for EAG recordings from antennae of intact, mechanically immobilized moths. Antennal responses were amplified (signal conditioner CyberAmp 320, Axon Instruments), digitized (Metrabyte DAS-16 A/D, sample period 500 ms) and analyzed by a Stand Alone Acquisition System PC software (Run Technologies).

The main pheromonal component **1** and analogues **2–6** were dissolved in hexane forming a series of dilutions from 0.1 ng to 1 μg per μl. Five ml aliquots were pipetted onto filter paper discs (10 mm, Whatman No. 2) and each loaded disc was inserted into a Pasteur pipette after evaporation of hexane. Stimuli were delivered on the antennal preparation by air puffs blown through the cartridge positioned 2.5 cm from the antenna. Stimulus duration was 0.8 s at air flow 1 l min^{-1}. A continual stream of clean and humidified air (1 l min^{-1}) was blown over the antennal preparation between successive stimulations. Intervals between stimuli ranged from 1 to 20 min depending on the type and intensity of the stimuli. Nine EAG replications on three male antennae were recorded for each serial dilution of each compound. The main pheromone component **1** at a dose of 50 ng served as a standard to normalize EAG responses from different individuals and to monitor viability and constancy of the preparation. Stimulation with the standard both preceded and followed each experimental session. The dose response curves were expressed as a percentage of the EAG response relative to the standard. Stimuli doses were corrected for differences in volatility relative to **1** and expressed in pmol units. The EAG responses to pheromone-analogue blends (1 : 1, 1 : 10 and 1 : 100 loaded on the same filter paper) were recorded under identical conditions. 1 : 1, 1 : 10, and 1 : 100 ratios of each pheromone-analogue blend were tested on the same antenna. Five replications were made using a new moth.

b) *Single sensillum recordings (SSR).* Receptor potentials and nerve impulses were recorded extracellularly from receptor cells associated with the *sensilla trichodea* using a modified tip-cutting technique[19]. The same instruments as for EAG recordings were used (AD sample period is 150 μs).

Behavioural Experiments

a) *Laboratory mating experiments*. The effect of analogues on OFM mating behaviour was investigated in disposable Petri dishes (i.d. 10 cm). The analogue was loaded on a filter paper disc (1 cm, Whatman No. 2) placed in the centre of the dish housing the male. After 10–15 min of equilibration a calling female was introduced into the dish. The behaviour of moths was observed for a 30-min period. Experiments were performed simultaneously on six dishes in four replicate series per each analogue. In control experiments, performed coincidentally with tests, the OFM mating behaviour was observed in six Petri dishes in atmosphere without any analogue. Mating efficiencies of males in the test and control dishes were expressed in the form of confusion coefficients, C.C. (%) = $(C_C/N_C - C_E/N_E)$. 100, where C.C. is the confusion coefficient, C_C and C_E represent a number of copulations in control and experimental groups containing N_C and N_E number of pairs, respectively.

b) *Wind-tunnel experiments*. OFM males were flown in a 1.86 × 0.3 × 0.3 m (L/W/H) Plexiglas wind-tunnel. Charcoal-filtered and humidified air was pushed through the tunnel by four ventilators. Air velocity was maintained at 0.5 m s^{-1}. The wind-tunnel conditions used were: 22–26 °C, 40–60% relative humidity and 700 lx light intensity. The experiments were performed from 3 to 1 h prior the beginning of scotophase. Males were released from a middle part of the tunnel into an odour plume which was created by pinning the filter paper disc (7 mm, Whatman No. 2) loaded with stimuli onto a holder placed centrally near the upwind end.

At the beginning of each flight session the standard pheromone mixture of *Z*8-12:OAc (90%), *E*8-12:OAc (6%) and *Z*8-12:OH (4%) at a total dose of 10 ng (this dose was found to be comparable with calling females in previous experiments) was presented to five males to control of their conditioning. If more than three males were activated, took oriented flight and found the source, new males were allowed to flight in response to pheromone-analogue blends. Two types of pheromone-analogue blends were tested: (1) a mixture of the respective analogue (90%), *E*8-12:OAc (6%) and *Z*8-12:OH (4%) and (2) a mixture of 10 ng of the standard pheromone blend and 100 ng of respective analogue (loaded on the same filter paper disc). Five males were tested for each filter paper source. In six replicate series, altogether 30 males were flown for each treatment. Male behaviour was classified into four categories: (i) activation (walking and wing fanning), (ii) taking off, (iii) oriented flight, and (iv) landing, touching the odour source and copulation attempts. The total time of observation was either two minutes if the male did not take off, or until it landed.

Statistical Analysis

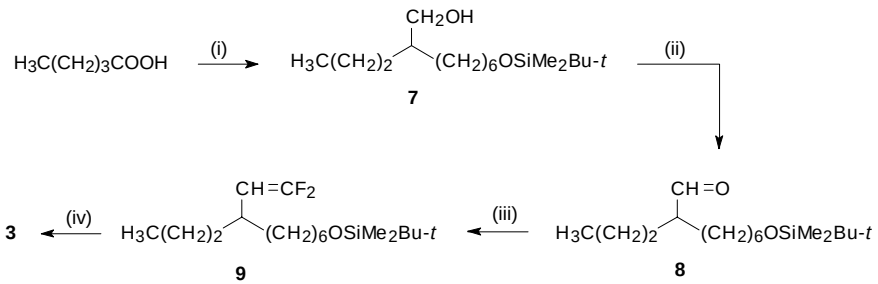
All data were evaluated by analysis of variance (ANOVA). Where the differences among treatments were noted, comparisons were made using Duncan's multiple range tests[20] at $P = 0.05$.

RESULTS AND DISCUSSION

The 2,2-difluoro analogue **3** was prepared using the strategy shown in Scheme 1. Thus, aldehyde **8** was prepared by alkylating the pentanoic acid dianion[14] with protected ω-bromoalkanol followed by a reduction ($LiAlH_4$)/oxidation (PCC) sequence[15]. The 2,2-difluorovinyl group was introduced using the Hayashi procedure[16] affording the protected alcohol **9** in a reasonable yield (38%). The thia analogues were conveniently prepared from commercially available ω-bromoalkanols of appropriate length by treating them with either sodium propane- or butanethiolates at 80 °C in DMF (Scheme 2). Thia alkanols were subsequently acetylated *in situ* to acetates **5** and **6**. The prepared

analogues were fully characterized by elemental and spectral analyses. The purities of samples used for biological tests were in all cases higher than 99% (capillary GC).

Dose-response curves constructed from EAGs of male *C. molesta* for **1** and its analogues **2–6** (Fig. 1) show significant differences in activity of the respective compounds. Particularly, there is a marked difference between thia and branched analogue groups; while the former (thia analogues **5** and **6**) is characterized by relatively high activity and similarity in the dose-response curves, a weak activity is generally shown by the group of branched analogues **2–4**. The saturation of EAG responses (dependent on the number of the receptor sites and the intrinsic activity of the particular compound) was lower than that for **1**, but well demonstrated for the thia analogues. On the other hand, any saturations were hardly observable for the branched analogues. Also,



(i) 1. $LiN(iPr)_2$, THF; 2. $Br(CH_2)_6OSiMe_2Bu$-*t*; 3. $LiAlH_4/Et_2O$; (ii) PCC, CH_2Cl_2, NaOAc; (iii) CF_2Br_2, HMPT, Zn, DMF; (iv) 1. Bu_4NF, THF; 2. Ac_2O, pyridine, DMAP

SCHEME 1

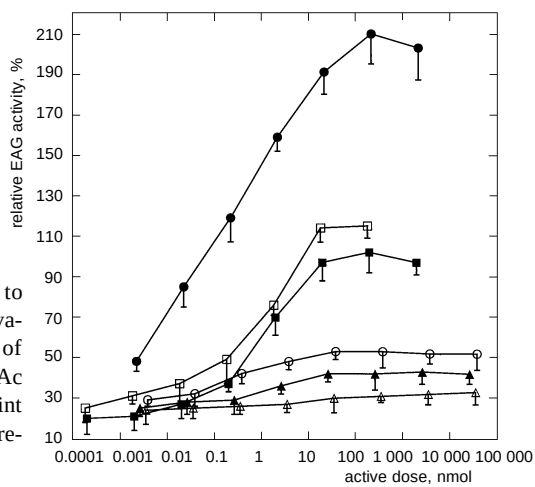
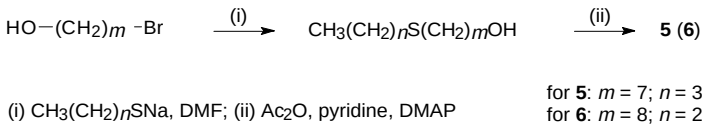


FIG. 1

Relative EAG responses of OFM males to compounds **1–6** corrected for differences in vapour pressures and expressed as percentage of EAG response to 50 ng dose of *Z*8-12:OAc (**1**). ● **1**, ❍ **2**, Δ **3**, ▲ **4**, ■ **5**, ❒ **6**. Each point is the mean of 9 replications. Error bars represent standard error of means

the concentration eliciting a half maximum relative EAG response (which is a measure of receptor sensitivity) was approximately the same for **1**, **5** and **6** but not for the branched analogues. The 2,2-difluorovinyl compound **3** possessed the lowest EAG activity in the series, indicating that introduction of fluorine atom into the vinyl group in **3** further decreased its activity in a manner similar to that observed for codlemone analogues bearing two or four fluorine atoms on double bonds[21] and also for fluorinated alkyl chain pheromone analogues[22].



SCHEME 2

The EAG responses elicited by 1 : 1 (w_t/w_t) mixtures of **1**/analogue (Fig. 2) were not significantly different from the response elicited by the standard dose of **1** (100%). At ten times higher doses of the analogues in the blend, **2**, **3** and **4** elicited EAG responses comparable with pure **1**, while the EAG responses to both **1**/**5** and **1**/**6** mixtures were significantly higher than that of **1** alone. The EAG responses to 1 : 100 **1**/**2** and **1**/**3** blends were significantly lower than that for **1**, while the increased responses of **1**/**5** and **1**/**6** mixtures were still well pronounced. These results indicate a competition between **1** and vinyl-branched analogues on *Z*8-12:OAc cells. It appears that either **1**, **2** and **3** bind to the same site of the receptor, or, at least, **2** and **3** interfere with the binding of **1**. As regards the increased EAG responses observed for the thia analogues **5** and **6** in EAG blend experiments, there are two possible interpretations: (i) due to their conformation flexibilities (see further discussion), **5** and **6** may also stimulate receptor types other than *Z*8-12:OAc, for instance those sensitive to *E*8-12:OAc, the minor pheromone com-

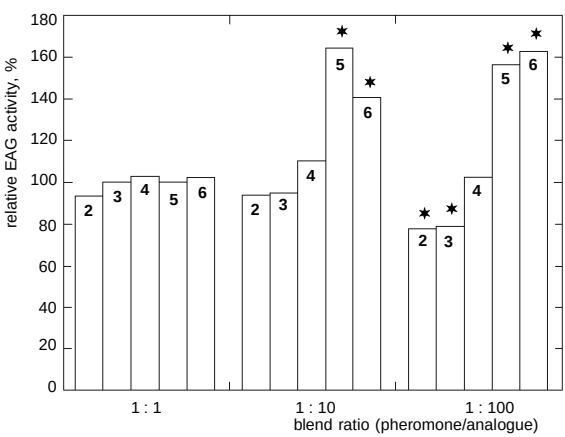


FIG. 2
Relative EAG activity of binary **1**/analogue mixtures (100 ng/100 ng–10 μg) on OFM males antennae. The 100% of EAG activity represents an average response for pure **1** at 100 ng dose. Bars represent the mean response of 9 separate replications; bars with asterisk are significantly different at $P = 0.05$ (Duncan's multiple range test)

ponent or those tentatively anticipated for the 12:OAc (refs[10,23]); and (ii) since **5** and **6** are quite potent stimulants for the *Z*8-12:OAc cell, increased EAG responses to 1 : 10 and 1 : 100 **1**/**5** and **1**/**6** blends may simply reflect an increased stimulation intensity.

The above suggestions (that **5** and **6** may also stimulate receptor types other than *Z*8-12:OAc) are, at least to a certain degree, corroborated by the MM+ calculations. Figure 3a illustrates the superposition of the minimum-energy conformer of 3-ethylhex-1-ene (modelling our vinyl analogues **2** and **3**) with calculated transoid conformer of (*Z*)-oct-4-ene. Although the aliphatic part of the model partially mimics the pheromone-like structure, the double bond of (*Z*)-oct-4-ene is not well fitted by the vinyl group in the model. For thia analogues (based on the ethyl propyl sulfide model) the S–CH_2 bond (1.79 Å) was found to be by 25% longer than the CH=CH double bond (1.34 Å) and the CH_2–S–CH_2 valence angle (96°) was found to be smaller than the CH=CH–CH_2 angle (126°) in (*Z*)-hex-3-ene. Further, ethyl propyl sulfide shows much higher flexibility than it was observed in the alkene, where the configuration is fixed by the double bond geometry and only two conformers can be calculated. We were able to find 27 ethyl propyl sulfide conformers encompassing energy from 13.51 to 27.49 kJ mol^{-1}. After applying the molecular dynamic procedure at 300 K, this number dropped to 10. The conformer showing the highest population at room temperature (300 K) was used for comparison with (*E*)- and (*Z*)-hex-3-enes (Figs 3b and 3c).

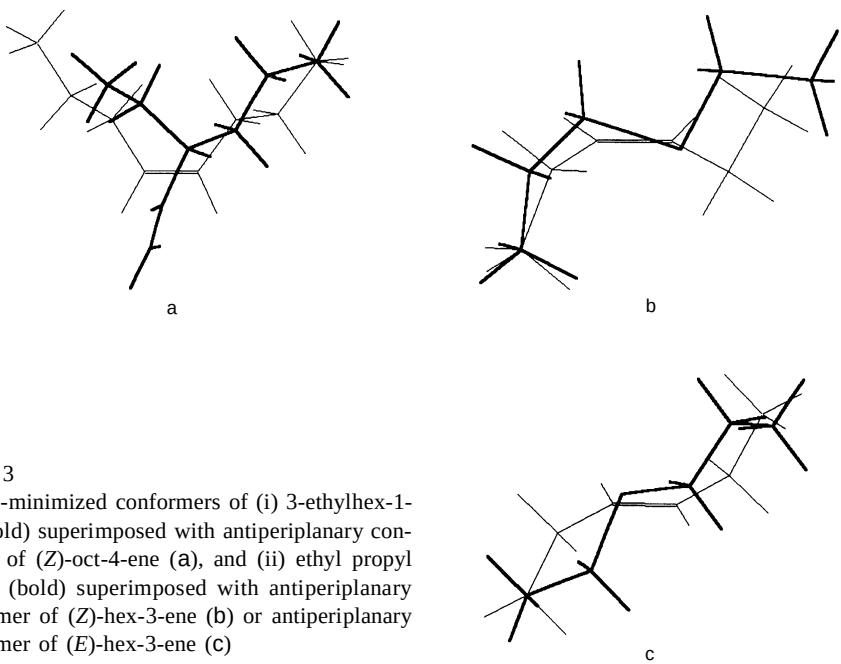


FIG. 3
Energy-minimized conformers of (i) 3-ethylhex-1-ene (bold) superimposed with antiperiplanary conformer of (*Z*)-oct-4-ene (a), and (ii) ethyl propyl sulfide (bold) superimposed with antiperiplanary conformer of (*Z*)-hex-3-ene (b) or antiperiplanary conformer of (*E*)-hex-3-ene (c)

In accord with experimental data, the foregoing computations indicate that (i) the –CH=CH– group in pheromone-like monoene compounds can be replaced by the $–CH_2–S–$ group in a rather bioisosteric way, and (ii) the thia analogues can retain an ability to interact with antennal proteins in a way similar to pheromones, although the differences in bond lengths and bond angles may result in not fully identical "packing" in the receptor protein cavity which is reflected by decreased EAG activity. The existence of a higher number of conformers may also decrease active concentrations of bonded structures due to the entropy factor.

The SSR experiments (Fig. 4) confirmed that both **1** and analogues **2–6** are sensed by the same cell. These recordings were performed at approximately equipotent concentra-

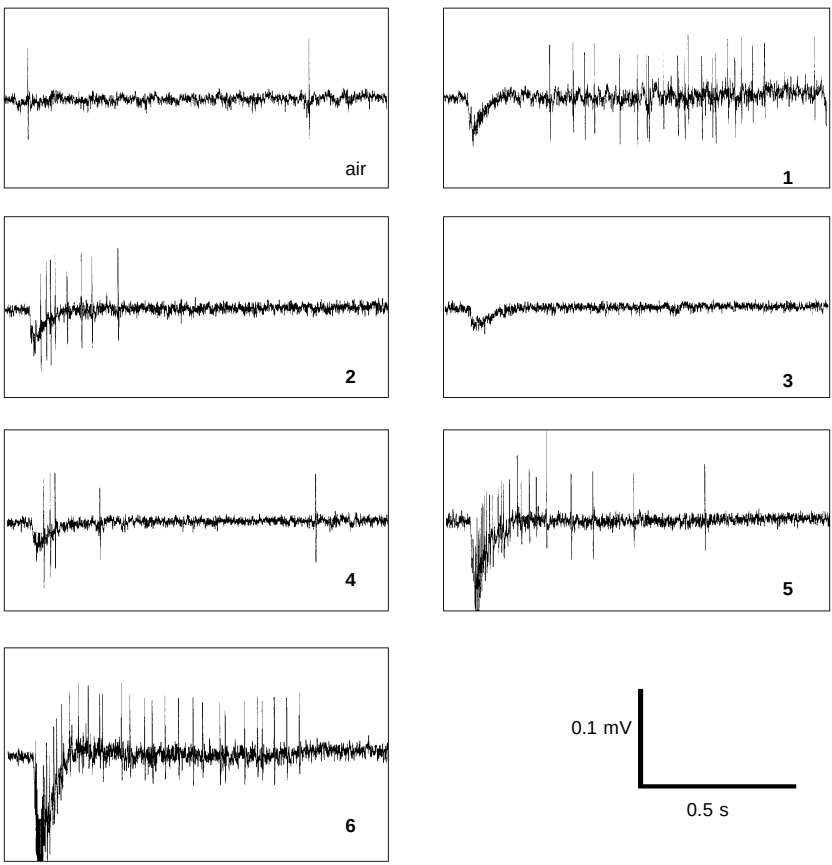


FIG. 4
Typical neural activity recorded from a single *s. trichodeum* on antennae of the OFM males in response to: air, **1** (5 ng), **5** and **6** (500 ng) and **2–4** (50 μg)

tions, using 5 ng (compound **2**), 500 ng (thia analogues **5** and **6**) and 50 μg (compounds **2–4**) doses. The SSR activity order **1** > **5** > **6** > **2** > **4** > **3** approximately follows the EAG values (DC changes measured from *sensilla trichodea* and the number of spikes). Similarly to **1**, the thia analogues **5** and **6** elicited spike activity during the whole period of stimulation, and even after its removal. On the other hand, the receptor responses to branched analogues were generally very weak and spikes, if any, were observed only during the onset of stimuli.

Taking all the electrophysiological data into consideration, one can suggest that the thia analogues behave as partial agonists while the branched analogues rank rather among competitive antagonists.

In the short-range behavioural assay within the Petri dish, all tested analogues had an inhibitory effect on the mating behaviour of OFM pairs (Fig. 5). As expected, the most potent "inhibitor" of copulatory behaviour was the pheromone component **1** exhibiting *ca* 45% inhibitions at both 1 and 10 ng doses and 75% at 100 ng, the highest dose tested. The thia compound **5** was the best inhibitor among the analogues inhibiting 70% of OFM pairs at 1 000 ng. At the same dose, the remaining analogues **2**, **3**, **4** and **6** showed 25, 45, 10 and 58% inhibition, respectively. Due to its very low inhibitory effect, the ethylidene analogue **4** was ignored in subsequent wind-tunnel experiments.

A similar order of inhibition, *i.e.* **1** > **5**, **6** > **2**, **3**, was observed in the wind-tunnel. In experiments where the standard (three component) pheromone blend was presented together with 100 ng of the respective analogue, we observed (Table I) that compounds **5** and **6** had a considerable inhibitory effect on male orientation in an odour plume. As a consequence, 70 and 92% of males did not locate the source. A similar inhibition (80%) was observed if the same dose (100 ng) of **1** was added to the standard pheromone blend. Under equivalent conditions, vinyl analogues **2** and **3** showed 40 and 45% inhibition, respectively.

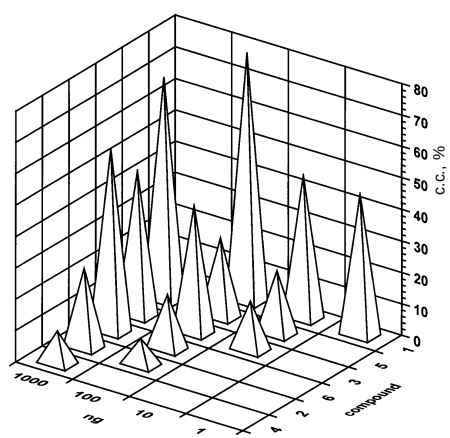


FIG. 5
Confusion of OFM pairs in an atmosphere within Petri dish permeated with the main pheromone component **1** and analogues **2–6** at gradually elevated doses. Each bar is the mean from 18 replications. The standard deviations were lower than or equal to 22%

All analogues had negligible "pheromonal" activity in experiments where **1** was replaced by respective analogues in the standard synthetic pheromone blend. Only the thia analogues **5** and **6** were able to substitute the main pheromone component **1**. Ten per cent of males were activated, 4% took oriented flight and no males found the odour source.

Although both types of analogues produced inhibitory effects on male sexual behaviour, the physiological processes mediating this inhibition appear to be quite different. High concentrations of **5** and **6** in behavioural experiments could (similarly to **1**) elicit a receptor overdischarge with subsequent neuronal adaptation, central habituation and/or a perceptual change in the signal quality[11]. In contrast, vinyl-branched analogues inhibiting the pheromone perception at the receptor level may produce an aberrant and/or insufficient sensory response which is not fully recognized as a proper sexual signal. Our results as a whole corroborate the suggestion[24] that structure requirements for inhibition of pheromonal response are far less restrictive than those for elicitation of that response. As regards the thia analogues **5** and **6**, the behavioural tests demonstrated that the thia analogues have a strong inhibitory effect on OFM male mating at 10 times higher doses (w_t) than the synthetic pheromone blend. If we additionally take into account the correction for differences in volatility, the analogues **5** and **6** show a significant inhibitory effect at concentrations almost comparable with those corresponding to the synthetic pheromone. Since the thia analogues can be easily prepared and their environmental stability probably exceeds that of the pheromone, they seem to have a potential in refining existing[25–27] insect pest control strategies based on mating disruption with sex pheromone.

TABLE I
Inhibition of behavioural responses of male *Cydia molesta* by mixtures containing the pheromone (10 ng) and a 10-fold excess of the compound tested

Compound	Inhibition of flight response[a], %			
	fanning	take off	oriented flight	touch/landing
1	40 d	53 c	73 b	80 e
2	0 a	0 a	32 ab	40 bc
3	3 abc	14 ab	45 b	45 bcd
5	0 a	0 a	32 ab	70 cde
6	0 a	0 a	33 ab	92 e

[a] Each value represents a mean of 6 replications. Different letters in columns indicate means that differ from the response elicited by 10 ng of the standard pheromone mixture (*Z*8-12:OAc, 90%; *E*8-12:OAc, 6%; *Z*8-12:OH, 4%) at the $P \leq 0.05$ level of significance (Duncan's multiple range test).

CONCLUSIONS

Although the details of inhibitory effects of analogues **2–6** on the male behaviour remain still speculative at present, our results demonstrate that two different mechanisms may be operative for vinyl-branched and thia analogues: the branched analogues appeared to be pheromone-antagonists, *i.e.*, their inhibitory properties are not entirely associated with their pheromone-mimicking capabilities. The observation that some vinyl analogues have a capability of inhibiting EAG responses to the pheromone at the antennal level is interesting and deserves further investigation. On the contrary, our data suggest that the replacement in the pheromonal carbon–carbon double bond by the $–CH_2–S–$ moiety resulted in pheromone agonists possessing the ability to rather modify than inhibit the pheromone perception. This effect is most likely caused by the ability of flexible thia analogues to interact with both major and minor pheromonal component receptors on OFM males antennae. The new analogues described may also prove useful as tools in further biochemical, and field mating disruption studies.

Financial support from the USAID (U.S. Agency for International Development), Program in Science and Technology Cooperation, grant No. DHR-5600-G-00-1051-00, is acknowledged with pleasure.

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