

New Mimics of the Acetate Function in Pheromone-Based Attraction

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Abstract—Several analogues of (Z)-8-dodecenyl acetate (**1a**), the major pheromone component of the Oriental fruit moth, *Cydia molesta*, with chloroformate and lactone functional groups in place of the acetate moiety, were synthesized and investigated for their biological activity at four evaluation levels, i.e. by electroantennography (EAG), electrosensillography (ESG), short-range sexual stimulation and activation in the flight-tunnel. We found very strict requirements on the shape as well as on the electron distribution of the acetate group for a productive interaction with the receptor. The behavioral results showed that, among the analogues investigated, the chloroformate **1b**, alken-4-olide **2a** and also dodecyl acetate (**1c**) possess significant (60–85%) inhibitory activities. Based on electrophysiological evidence demonstrating that (i) only **1b** is competing with the major pheromone component **1a** for the same receptor sites on the male antennal *sensilla*, (ii) **1c** elicits moderate EAG but no ESG responses and (iii) **2a** does not produce any electrophysiological response at all, three possible inhibitory mechanisms by which these analogues are acting could be distinguished. Copyright © 1996 Elsevier Science Ltd

Introduction

The mating disruption technique belongs to one of the most perspective strategies for application of pheromones and their analogues in insect pest control.^{1,2} To achieve the mating disruption effect, several basic approaches are possible,³ including: (i) the release of synthetic pheromone constituents or mixtures thereof into the environment to disrupt the male's ability to locate virgin females by the omnipresence of pheromone, (ii) the use of components emitted by closely akin species to reduce intraspecific attraction and (iii) the use of reactive pheromone mimics or inhibitors that either irreversibly and stoichiometrically block antennal receptor proteins or specifically inactivate pheromone catabolizing enzymes. To date, however, no evidence suggests that the last two approaches are as efficacious in disruption as synthetic copies of the natural pheromone and no operational system based on any of these principles has been invented. Nevertheless, investigations concerned with modifying effects of various chemicals on insect olfaction indicate that some substances are able to react with specific chemical group at binding proteins, receptor proteins and/or catabolic enzymes that comprise the olfactory chemosensory systems of the moths. These compounds may be structurally related to a component of the pheromone of the insect against which they are applied, or they may be of an entirely different chemical class. For example, Berger and Estes⁴ found that some *N*-alkyl-maleimides are able to irreversibly block the electroantennographic response of the cabbage looper *Trichoplusia ni* to the pheromone and to significantly

reduce the ability of males to mate. A similar effect has been observed⁵ on the exposure of olfactory organs of the male moth *Mamestra brassicae* to *N*-ethylmaleimide vapors. Also, some trifluoromethyl ketones have been proven to inhibit the action of pheromone esterases in the processionary moth *Thaumetopoea pityocampa*⁶ and the Egyptian armyworm *Spodoptera litoralis*.⁷

In this study, the males of the Oriental fruit moth (*C. molesta*), CM, were selected as model species. The female sex pheromone includes (Z)-8-dodecen-1-yl acetate (**1a**) as the main constituent⁸ of the three-component mixture. We describe herein the synthesis and biological activity in electrophysiological and behavioural assays of analogues **1b–2** (Scheme 1). These analogues formally proceed from structural modifications of the acetate group, one of the three putative active sites of the parent molecule **1a**, which could be involved in the interaction process with the antennal proteins. Due to the suggested⁹ bioisosterism of CH₃⇒Cl replacements and some type of similarity existing between the acetate and lactone functions, these analogues seemed to embody in their structures both the appropriate recognition and reactivity elements. It was anticipated that processing some of the analogues by a target protein could result in unmasking the inherent latent reactive moiety in **1** and **2**, and lead to inactivation of target acetate-recognizing antennal proteins. Based on some earlier findings related to the inhibition of esterases by propan-3-olide derivatives,^{10,11} we hypothesized that some of the compounds represented by structures **2** might function as inhibitors of pheromone carboxylesterases. In view



In formulae

1a: R = (Z)-7-undecenyl; Y = CH₃
1b: R = (Z)-7-undecenyl; Y = Cl
1c: R = *n*-undecyl; Y = CH₃
1d: R = *n*-undecyl; Y = Cl

2a: R = (Z)-7-undecenyl; n = 1
2b: R = (Z)-7-undecenyl; n = 2
2c: R = (Z)-7-undecenyl; n = 3

Scheme 1.

of the reported¹² inhibitory activity of dodecyl acetate (**1c**) on the CM males under field conditions, we also included the saturated pheromone analogues **1c** and **d** into our studies.

Results and Discussion

Chemistry

Chloroformates **1b** and **1d** were prepared from the corresponding alcohols and triphosgene, a safe and crystalline substitute of phosgene, according to a known¹³ procedure. Compounds **2a** and **c** were synthesized as shown in Schemes 2 and 3, while the synthesis of **2b** has been described previously.¹⁴ IR, NMR and elemental analyses of all synthesized compounds were fully consistent with the proposed structures.

Relative volatiles of analogues

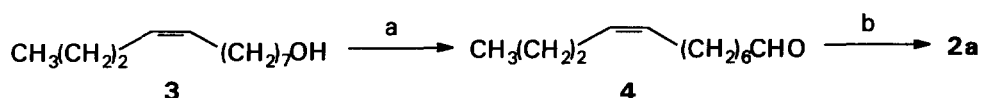
Dose-response curves in insect electrophysiology and results of laboratory bioassays are usually based on amounts of stimulus applied to the odor source rather than the amount of stimulus to which the insect antenna is actually exposed. While such approach may be correct when the compounds to be compared are structurally similar and hence of (approximately) the same volatility, it is certainly not valid for compounds differing significantly in their chemical characteristics and molecular mass. This implies that corrections for differences in volatility need to be taken into account when interpreting electrophysiological and behavioral results for analogues **1** and **2**. Since the potential volatility of a chemical is related to its inherent vapor pressure,¹⁵ we determined the saturated vapor pressures of **1** and **2** using a GC method, whose details have been described previously.^{16,17} Table 1 lists the

relative GC retention times of the compounds measured isothermally at five temperatures along with the calculated vapor pressure data. The results impressively demonstrate the importance of vapor pressure corrections in comparing electrophysiological and behavioral data for **1** and **2**: at 25 °C, the relative liquid-vapor concentration ratio for the pheromone component **1a** and analogues **1b**, **c**, **d**, **2a**, **b** and **c** follows the order 1:1, 1:0.61, 1:0.85, 1:0.45, 1:0.175, 1:0.027 and 1:0.0093, respectively. It means that the alken-5-olide (**2c**) is about two orders of magnitude less volatile than the pheromone component **1a**. Note that corrected concentrations of all analogues are used throughout this work.

Electrophysiological properties

Two types of electrophysiological recordings were conducted to assess the effects of the pheromone-related chemicals on antennae.

Electroantennography. Relative EAG activities of compounds **1a-d** and **2b** corresponding to 50% of the relative activity scale (Fig. 1) demonstrate that in no case did a pheromone analogue reach the response value of the natural pheromone component, (Z)-8-dodecen-1-ol acetate (**1a**). The corrected EAG activity of analogues decreases in the relative ratio 1400:15.1:3.1:1.8:1 for the **1a**, **b**, **2b**, **1c** and **d**, respectively. As expected, chloroformate **1b**, which is the closest structural relative of the natural pheromone component and, rather surprisingly, also the alken-4-olide **2b**, were the most active analogues. Their activity range was between 0.05 and 500 µg of the stimulus. Responses elicited by different doses of these two compounds are not significantly different (Student's *t*-test, $\alpha = 0.05$). However, at the upper dose used (500 µg), an olfactory saturation could be



Scheme 2. (a) Pyridinium chlorochromate/CH₂Cl₂; (b) ketene, BF₃·Et₂O/THF, -40 °C.

observed for **1b**, but not for **2b**. The last two compounds, **2a** and **c**, elicited nonsignificant responses when compared with the response of pure hexane (Student's *t*-test, $\alpha = 0.05$), even at the highest doses tested (500 μg).

Electrosensillography. The activities of olfactory neurons associated with *sensilla trichodea* were analyzed (Fig. 2). In several of the preparations, very small number of impulses was observed that could not be reliably discriminated from the spontaneous responses in air (Student's *t*-test, $\alpha = 0.05$). For this reason, these responses were not considered in further

analysis in this report. All neurons sensitive to **1a** were also responsive, but to a lesser extent, to **1b** and **2b**. Thus, the present data suggest that these compounds interact with the same receptor sites as the major pheromone component **1a**. With exception of the saturated acetate **1c**, the order of the activity of the compound tested paralleled their EAG activity.

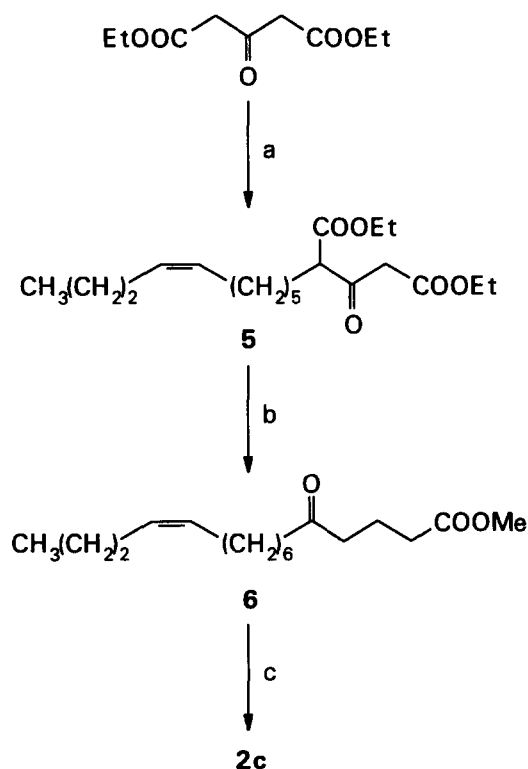
Short-range behavior

The effect of the analogues on short-range communication among the CM conspecifics is shown in Figure 3. The values of 'confusion coefficients' determined at four concentration levels (see Experimental) demonstrate that, of the compounds tested, the chloroformate (**1b**) and alken-3-olide (**2a**) possess a significant inhibitory effect on the mating behavior, while the saturated chloroformate (**1d**) and alken-5-olide (**2c**) elicit only a very weak inhibitory effect. Therefore, the last two compounds were ignored in further flight-tunnel experiments. None of the analogues were found to be as active as the moth's own main pheromone component **1a** in causing disruption of normal pheromone-induced behavior.

Flight-tunnel observations

Of the analogues tested, the chloroformate **1b** was to some extent able to substitute for the main pheromone component **1a** in the pheromone blend, although it did not elicit the whole behavioral sequence (15% of males were activated and took flight, none of them orientated to the source or exhibited a precopulatory behavior). Similar, but even less pronounced effect was observed for **1c** and **2b**.

The behavior profiles presented in Figure 4 indicate that the exposure of the CM males to a mixture of the pheromone (10 ng of the three-component blend, which was proved to be comparable with calling females) and a 10-fold excess of the analogues **1b**, **c** and **2a** resulted in a significant reduction (60–85%) in touch/landing responses relative to the pheromone



Scheme 3. (a) (i) $\text{Mg}(\text{OEt})_2/\text{EtOH}$, (ii) $(Z)\text{-CH}_3(\text{CH}_2)_2\text{-CH=CH-(CH}_2)_5\text{Br}$; (b) (i) $\text{NaH}/\text{MeO}(\text{CH}_2)_2\text{OMe}$, (ii) $\text{Br}(\text{CH}_2)_5\text{COOEt}/\text{NaI}$, (iii) decarboxylation/hydrolysis, (iv) $\text{CH}_3\text{N}_3/\text{Et}_2\text{O}$; (c) (i) $\text{NaBH}_4/\text{EtOH}$, (ii) $\text{H}^+/\text{CH}_2\text{Cl}_2$ reflux 24 h.

Table 1. GC vapor pressures of **1** and **2**

Compound ^a	Relative retention times							Vapor pressures	
	60 °C	70 °C	80 °C	90 °C	100 °C	110 °C	120 °C	p_{GC} (Pa)	p_a/p_p
1a	4.105	3.802	3.509	3.265	3.066	—	—	0.312 ^b	1.0
1b	2.560	2.410	2.320	2.230	2.130	—	—	0.184 ^c	0.59
1d	1.330	1.31	1.290	1.280	1.260	—	—	0.135 ^d	0.43
2a	—	2.770	2.660	2.560	2.480	2.400	2.310	0.0548 ^d	0.176
2b	—	—	1.956	1.912	1.877	1.837	1.816	0.00828 ^e	0.027
2c	—	—	4.054	3.808	3.644	3.432	3.329	0.00281 ^e	0.0090

^a Experimental values for **1c** in ref. 17.

^b Standard *n*-tetradecane.

^c Standard *n*-pentadecane.

^d Standard *n*-hexadecane.

^e Standard *n*-octadecane.

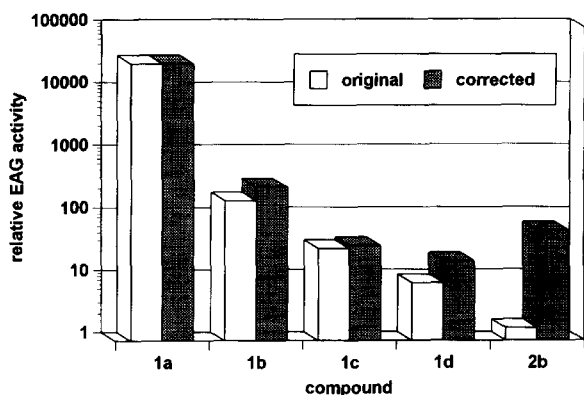


Figure 1. Experimental EAG activities for compounds **1a–d** and **2b**; a comparison of uncorrected and vapor pressure-corrected data. The activities are expressed as the reciprocal of the relative number of μmol required to elicit the same receptor response. Each bar represents the mean of at least six experiments SD did not exceed 11%.

alone. Under the same conditions, the addition of **2b** to the pheromone did not significantly affect the percentage of males flying upwind and contacting the odor source. At a lower concentration ratio (1:1) even the analogues **1b**, **c** and **2a** were only slightly effective. When the behavioral spectrum of males responding to pheromone is compared with the behavior of males responding to a pheromone/analogue (1:10) mixture, the most affected maneuver was oriented flight: activated males were less successful in their orientation in the odor plume. Males that found the source eventually needed a longer period than the males responding to the pheromone.

The neurophysiological data presented here indicate that there is a class of receptor neurons on the antenna of CM male that significantly responds to stimulation with the major pheromone component **1a** and to stimulation with two other analogues, **1b** and **2b**. Nonuniform spike activities produced by the analogues **1c** and

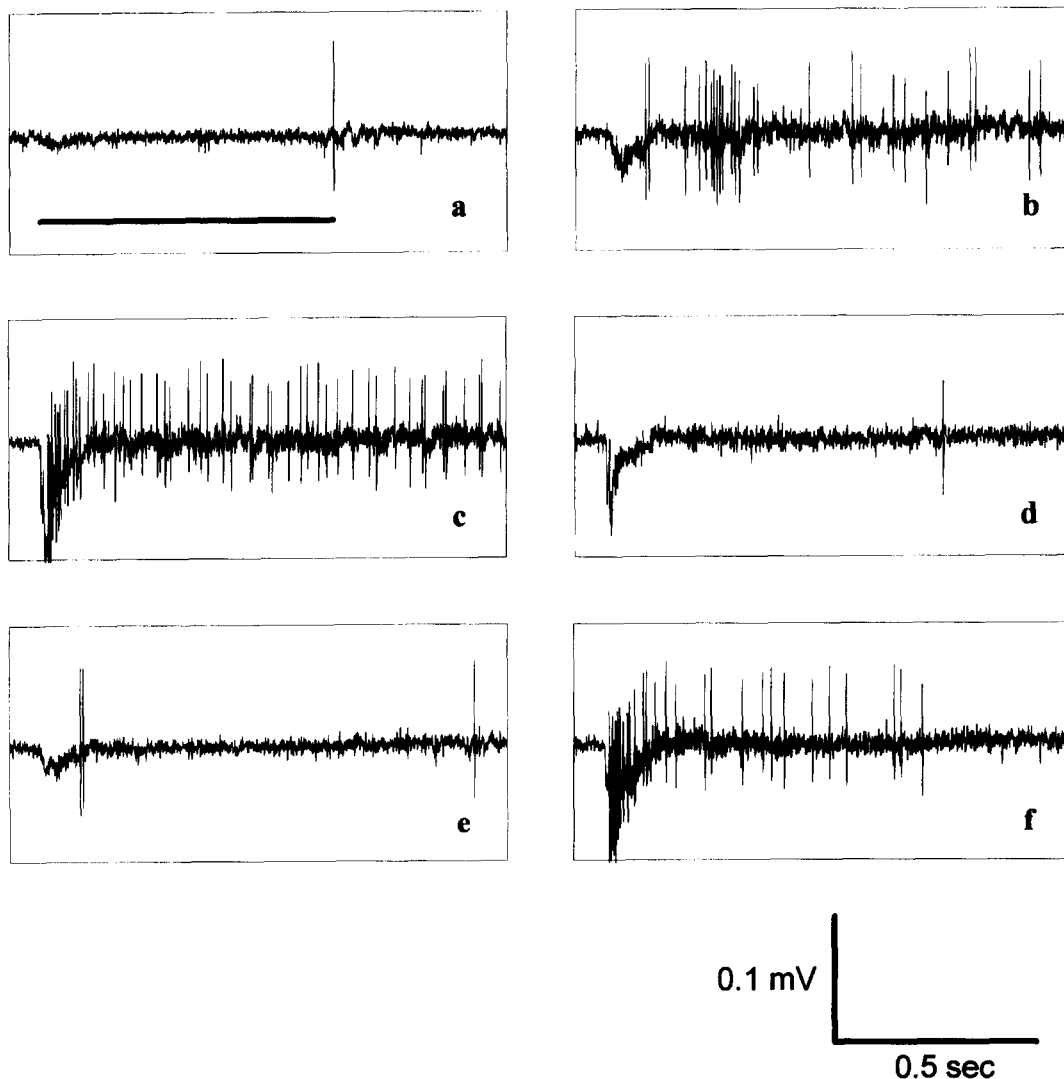


Figure 2. Typical neural activity recorded from a single *s. trichodeum* on antennae of the Oriental fruit moth in response to: (a) air, (b) $5 \mu\text{g}$ of **1a**, (c) $50 \mu\text{g}$ of **1b**, (d) $50 \mu\text{g}$ of **1c**, (e) $50 \mu\text{g}$ of **2a** and (f) $50 \mu\text{g}$ of **2b**. The bar below the recording (a) indicates the duration of the olfactory stimulation (0.8 s).

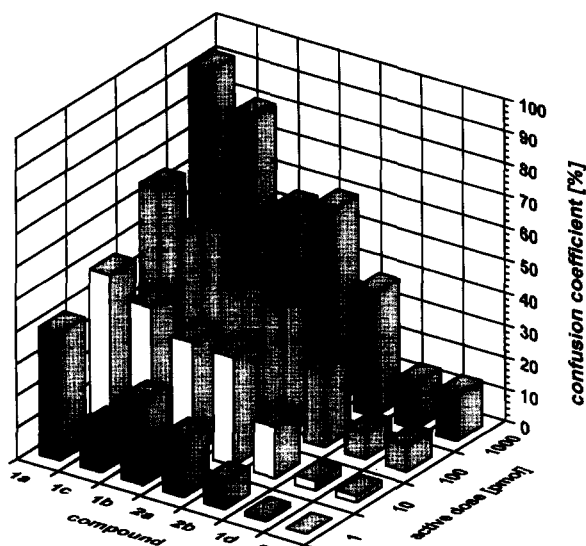


Figure 3. Inhibition of male *C. molesta* behavior in a laboratory short-range bioassay promoted by different doses of **1** and **2**. Each bar is the mean of 18 experiments ($SD \leq 15\%$).

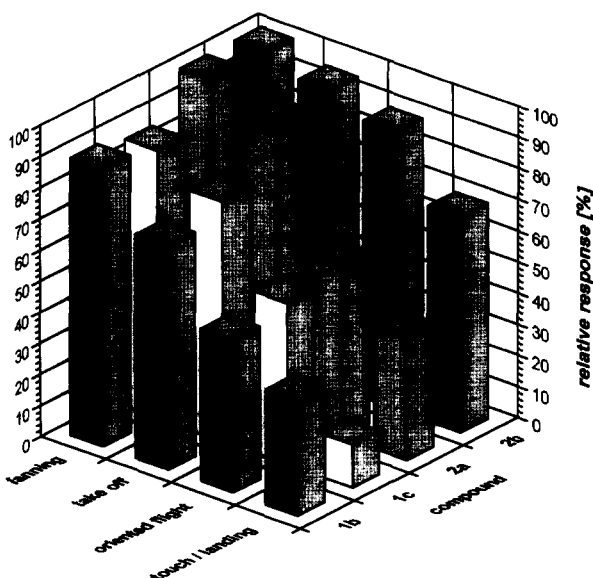


Figure 4. Inhibition of behavioral responses of male *C. molesta* by mixtures containing the pheromone (10 ng) and a 10-fold excess of the respective analogue, relative to male responses to the pheromone alone. Each bar is the mean of six experiments ($SD \leq 18\%$).

2a did not significantly differ from the spontaneous ones. Analogues **1d** and **2c** did not elicit any responses, even when high concentrations were used. A rather good qualitative correlation was observed between our laboratory short-range assay data and the flight-tunnel results (Figs 3 and 4). The most interesting feature of the results are the striking differences among analogues in the complementary electrophysiological and behavioral tests. As illustrated in Table 2 summarizing the qualitative output of these tests for the most important analogues of the series, none of the analogues **1b**, **c**, **2a** and **b** showed identical behavior at all four (EAG, ESG, short-range behavioral assay and flight tunnel) levels of evaluation. This implies that no straightforward structure–activity relationship can be discerned and the inhibitory active analogues **1b**, **c** and **2a** must operate by (at least) three different inhibiting mechanisms. Although, at present, we have no evidence of the exact mode of action of the analogues tested, several mechanisms can be hypothesized.

The mechanisms of inhibitory action derive from inhibiting chemicals — in relation to the pheromone composition of the species. It is generally accepted¹⁸ that molecular size and shape are important for insect pheromone chemoreception. Apart from stereochemical requirements, however, electronic charge–charge attraction, hydrogen bonding, hydrophobic bonding and van der Waals forces are potentially important in binding to proteinaceous macromolecules. To account for the surprising differences in biological activity of the analogues, we first considered the possibility that some of the analogues might assume minimum energy conformations that were very different from the conformation of **1a**. As the molecular shapes of compounds **1a,b** and **2a,b** are identical with respect to the unsaturated hydrocarbon chain, the main spacial differences among them should originate from the polar groups. Thus, the energy-minimized molecular geometries of ethyl acetate has been superposed on those of ethyl chloroformate, butan-3-olide and pentan-4-olide to qualitatively compare the shapes. The results are shown in Figure 5. The chloroformate [Fig. 5(a)] and, to a lesser extent, also the alken-4-olide [Fig. 5(c)] show clearly a high degree of similarity to the acetate group. On the contrary, the alken-3-olide [Fig. 5(b)] shows the largest deviations. Accordingly, it is more probable that the receptor would reject the four-membered ring due to its size or shape.

Table 2. Qualitative summary of responses^a for male *C. molesta* on exposure to compounds **1a–c**, and **2a** and **b**

Compound	Electrophysiological activity		Inhibitory effect	
	EAG	ESG	Short-range bioassay	Flight-tunnel
1a	(+)	(+)	(+)	(+)
1b	(+)	(+)	(+)	(+)
1c	(+)	(–)	(+)	(+)
2a	(–)	(–)	(+)	(+)
2b	(–)	(+)	(–)	(–)

^a(+): positive response, (–): no or statistically non-significant (Student's *t*-test, $\alpha = 0.05$) response.

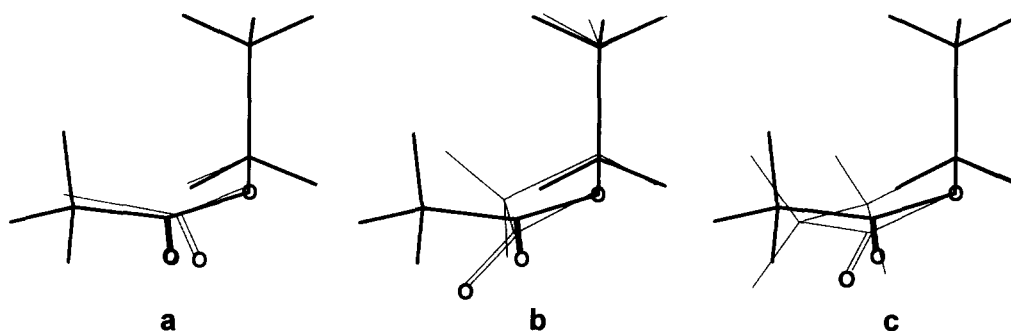


Figure 5. Superpositions of the energy-minimized ethyl acetate structure (bold structures) with those of (a) ethyl chloroformate, (b) butan-3-olide and (c) pentan-4-olide.

Although the replacement of the acetate methyl group by a chlorine in **1b** does not seem to have important steric consequences, the methyl group has a higher hydrophobicity than the chlorine atom and, also, the possibility to engage in short-range binding through dispersion forces with the receptor structure that is complementary to the acetate methyl is probably reduced for the chloro derivative.^{19,20} Beside this, the IR carbonyl frequencies $\nu(\text{C}=\text{O})$ for **1a** and **b** (1740 versus 1778 cm^{-1}) differ significantly, indicating the different ability to form the hydrogen bond. The hydrogen bond is widely regarded²¹ as being the most important intra- and intermolecular cohesive force and a major contributor of noncovalent interaction energy in biological systems. All these differences may account for the reduced electrophysiological activity of the chloroformate **1b** in comparison to **1a**. Similarly to our results, only moderate electrophysiologic responses were found²² to be evoked by formate mimics of the aldehyde function on antennal receptors of several noctuid species. Regardless of its reduced activity, the analogue **1b** elicits a sufficiently high electrophysiological response from the pheromone-sensitive receptor neurons and could theoretically overload the receptor system acting as an inhibitor when present in hyperphysiological concentrations. Another explanation for the significant inhibitory activity of **1b** could be its possible binding to antennal proteins through a carbamate linkage (under evolution of HCl!), thereby locking the sensory transduction mechanism and/or inactivating the pheromone catabolizing enzymes. Precedent for this analogy is found in the use of (Z)-11-hexadecenyl- and (Z)-9-tetradecenyl fluorides as reactive mimics of the *Heliothis virescens* pheromone aldehydes.²³

The fact that we found relatively high (and nearly equal) inhibitory activity to be coupled both with **1b** and **2a** was rather surprising since the alken-3-olide (**2a**) failed to produce any significant EAG or ESG response. If the specificity of pheromones and pheromone receptors was coupled to specificity of the pheromone clearing enzyme system, it would be expected that analogues that successfully mimic the pheromone should serve as better substrates for the pheromone-specific catabolic systems and be degraded more effectively than those compounds that are structurally less similar to the pheromone. Thus, compounds lacking

sufficient pheromone mimicry (e.g. **2a**) would be cleared from the receptor less effectively and act as behavioral inhibitors. The earlier studies of behavioral responses of male codling moths²⁴ and European corn borers²⁵ in a flight tunnel to their corresponding pheromones and analogues led to the conclusion that the steric requirements for an analogue to be a pheromone mimicking substance are much more stringent than for the analogue to be an inhibitor of behavioral output. On the other hand, it is known²⁶ that four-membered lactones as ambident electrophiles may undergo, in the presence of nucleophiles, oxygen-alkyl or oxygen-acyl bond cleavage. Therefore, **2a** might be able to inhibit pheromone-catabolizing enzymes via acylation or alkylation of their nucleophilic groups. This would, in turn, lead to disruption of normal pheromone-induced behavior via prolonging high pheromone levels within the peripheral sensory system. A lower inhibitory activity of the alken-4-olide (**2b**) (Figs 3 and 4) may in part be related to its lower (in comparison to **2a**) chemical reactivity. Note that propan-3-olide has been found²⁷ about 50–100 times more reactive in reaction with adenosine, cytidine or guanosine than butan-3-olide while butan-4-olide completely failed to react with any of these nitrogen nucleophiles. A double-bond environment appears essential for good inhibitory activity since **1d**, the saturated equivalent of **1b**, is only a very weak (Fig. 3) inhibitor.

The behavior of the saturated acetate **1c** deserves a special comment. The major component of the Oriental fruit moth pheromone, (Z)-8-dodecenyl acetate (**1a**), was identified²⁸ in 1969. Since 1979 it has been assumed⁸ that the Oriental fruit moth pheromone consists of four components, viz. (Z)-8- and (E)-8-dodecenyl acetates, (Z)-8-dodecen-1-ol and dodecanol. The insect attraction was found²⁹ to be particularly sensitive to both the pheromone component ratios and concentrations. Mating disruption was also attempted with an analogue of **1a**, dodecyl acetate (**1c**). For example, Rothschild found¹² that males of this insect were not trapped at live virgin female or synthetic pheromone sources when dodecyl acetate (**1c**) was present in large amounts (exceeding the amount of the unsaturated acetates) in the same trap or within 15 cm from the bait. When the compound, however, was present as a background odor over a large area, an opposite (synergistic) effect was

observed. Reasons for these results remained obscure. Recently, the composition of the pheromone blend was re-examined and significant amounts ($3.44 \pm 1.16\%$) of **1c** have been identified³⁰ in the effluvia of calling females. It has been suggested that the role of this compound in the natural pheromone of CM might have been overlooked. Our electrophysiological results demonstrating that **1c**, although not detected by the same receptor cell type as **1a**, elicits a relatively high EAG activity, seem to support this suggestion. Unfortunately, neither our own ESG experiment nor the previous ESG studies³¹ were able to detect any other receptor cells that could respond to **1c**, probably due to the small size of the moths. If the proposition is accepted that this compound represents a minor pheromone component and by itself could mediate a particular element of behavior, then a background of a high concentration of this component might alter the balance of sensory input to such an extent that the insects no longer respond appropriately. A similar inhibitory effect of imbalance in the pattern of sensory input had been previously observed³² on the behavior of *Autographa gamma* species that use a simple two-constituent pheromone blend. Increasing the level of minor component in the binary blend resulted in a substantially decrease of male responses in moth behavioral stages.

In conclusion, the present data suggest that, of the analogues investigated, only the chloroformate (**1b**) and the alken-4-olide (**2b**) are able to reasonably mimic the acetate function and thus produce responses from the same class of pheromone receptor neurons as the major pheromone component **1a**. The inhibitory properties of the analogues, however, seem not to be entirely connected with their mimicking capability. Apparently, several constitutional and configurational properties of the molecule and, in turn, its chemical reactivity are of special significance to the inhibitory process. More specific studies are required to elucidate the role these factors may play for effective binding to proteinaceous substrates. At present, the inhibitory mechanisms remain speculative. In spite of this, the new inhibitors described (especially **1b** and **2a**) may prove useful as tools in further biochemical as well as field studies directed towards mechanisms controlling mating disruption.

Experimental

Chemistry

¹H NMR spectra were determined in CDCl₃ solution on a Varian UNITY-500 spectrometer operating at 499.5 MHz and absorptions are expressed in δ (ppm) scale relative to TMS. The IR spectra were recorded on a Bruker IFS 88 FT-IR spectrometer in CCl₄. GLC analyses were performed on a Hewlett Packard HP 5880A chromatograph equipped with a FID detector and a 25 m capillary column (0.3 mm i.d., HP5-5% phenyl methylsilicone, cross-linked). 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 a stepwise gradient of ethyl acetate in light petroleum.

Syntheses

(Z)-8-Dodecen-1-yl chloroformate (1b). Triphosgene (0.367 mmol) solution in dry THF (1 mL) was cooled on an ice-bath and pyridine (45 μ L, 1.2 equiv.) was added. To the preformed white precipitate the (Z)-8-dodecen-1-ol (85 mg, 0.466 mmol) in THF (2 mL) was added dropwise during 20 min. After 2 h of being stirred at the ice-bath temperature, the reaction mixture was poured into ice and aq HCl (3.7%, 0.4 mL). Subsequent standard work up and PMPLC afforded the chloroformate **1b** (69 mg, 60% yield). Calcd for C₁₃H₂₃O₂Cl: C, 63.27; H, 9.39; Cl, 14.37. Found: C, 63.44; 9.50; Cl, 14.21. ¹H NMR: δ 0.90 (t, 3H, $J = 7.0$ Hz, CH₃CH₂CH₂—), 1.29 (m, 12H, 6 \times —CH₂—), 1.72 (m, 2H, $J = 4 \times 6.8$ Hz, —CH₂CH₂CO₂Cl), 2.01 (m, 4H, —CH₂—CH=CH—CH₂—), 4.31 (t, 2H, $J = 7.8$ Hz, —CH₂CO₂Cl), 5.38 (m, 2H, —CH=CH—). IR (cm⁻¹): 3006 m [ν (C—H), *cis*-double bond], 1778 vs [ν (C=O), ester], 690 w [ν (C—Cl)].

Dodec-1-yl chloroformate (1d). Calcd for C₁₃H₂₅O₂Cl: C, 62.76; H, 10.13; Cl, 14.25. Found: C, 62.90; H, 10.22; Cl, 14.14. ¹H NMR: δ 0.88 (t, 3H, $J = 7.0$ Hz, CH₃CH₂—), 1.26 (m, 18H, —CH₂—), 1.73 (m, 2H, $J = 4 \times 6.8$ Hz, —CH₂CH₂CO₂Cl), 4.31 (t, 2H, $J = 7.8$ Hz, —CH₂CO₂Cl). IR (cm⁻¹): 1779 vs [ν (C=O), ester], 690 w [ν (C—Cl)].

(Z)-8-Dodecan-1-al (4). Pyridinium chlorochromate (PCC; 3.5 g, 16.3 mmol) was suspended in dry CH₂Cl₂ (25 mL) and (Z)-8-dodecan-1-ol (**3**, 2.0 g, 10.9 mmol) in 6 mL of the same solvent was added in one portion to the stirred solution.³³ After 2 h, dry ether (50 mL) was added and the supernatant decanted from the black gum. The insoluble residue was washed with ether (3 \times 20 mL), the combined ethereal solution was passed through a short pad of neutral alumina with charcoal and the solvent was evaporated. PMPLC of the crude product afforded 1.64 g (83%) of the aldehyde **4**. Calcd for C₁₂H₂₂O: 79.05; H, 12.17. Found: C, 79.11; H, 11.98. ¹H NMR: δ 0.95 (t, 3H, $J = 7.0$ Hz, CH₃CH₂CH₂—), 1.21–1.43 (m, 8H, 4 \times —CH₂—), 1.44–1.71 (m, 2H, —CH₂CH₂CHO), 2.05–2.11 (m, 4H, —CH₂—CH=CH—CH₂—), 2.42 (dt, 2H, $J = 7.0$, 1.6 Hz, —CH₂CHO), 5.31–5.38 (m, 2H, —CH=CH—), 9.77 (t, 1H, $J = 1.6$ Hz, —CHO).

(Z)-10-Tetradecen-3-olide (2a). Ketene³⁴ was bubbled into the stirred mixture of aldehyde **4** (1.6 g, 8.8 mmol) and borotrifluoride etherate (26 μ L, 0.2 mmol) in anhydrous THF (10 mL) at -40°C .³⁵ After 1 h, another fresh catalyst was added (20 μ L, 0.16 mmol). When the starting aldehyde **4** disappeared (2 h), the solution was flushed with dry nitrogen. Anhydrous triethylamine (2 mL) and CHCl₃ (20 mL) were added

at -40°C followed by water (20 mL). The mixture was then extracted with CHCl_3 (3×20 mL) and the combined organic extracts were dried over MgSO_4 . Removal of the solvent in vacuo and purification of the residue by PMPLC afforded 0.99 g (54%) of the pure (GLC) lactone **2a**. Calcd for $\text{C}_{16}\text{H}_{28}\text{O}_2$: C, 76.14; H, 11.18. Found: C, 76.31; H, 11.21. ^1H NMR: δ 0.90 (t, 3H, $J = 7.0$ Hz, $\text{CH}_3\text{CH}_2\text{CH}_2-$), 1.21–1.47 (m, 10H, $5 \times -\text{CH}_2-$), 1.62–1.85 (m, 2H, $-\text{CH}_2-\text{CH}-$), 2.02–2.12 (m, 4H, $-\text{CH}_2-\text{CH}=\text{CH}-\text{CH}_2-$), 3.06 (dd, 1H, $J = 16.2/4.3$ Hz, $-\text{CH}_2-\text{CO}-$), 3.51 (dd, 1H, $J = 16.2/5.8$ Hz, $-\text{CH}_2-\text{CO}-$), 5.32–5.39 (m, 2H, $-\text{CH}=\text{CH}-$). IR (cm^{-1}): 3004 m [$\nu(\text{C}-\text{H})$, *cis*-double bond], 1836 vs [$\nu(\text{C}=\text{O})$, lactone], 1653 w [$\nu(\text{C}=\text{C})$].

Diethyl 4-[(Z)-6-decenyl]-3-oxopentanedioate (5). To a stirred ethanol solution (150 mL) of the magnesium chelate prepared from diethyl 3-oxopentanedionate (12.7 g, 63 mmol), magnesium turnings (2.29 g, 95 mmol) and a trace of iodine was added 1-bromo-(Z)-6-decene (18.0 g, 75.5 mmol) at room temperature and the mixture was refluxed for 18 h under argon.³⁶ The reaction mixture was evaporated in vacuo to give a dark oil which was acidified with 10% HCl and taken up into ether. The ethereal solution was washed with water and dried over MgSO_4 . After evaporation, chromatography (PMPLC) of the crude product gave 17.2 g (80%) of the oxoester **5**. Calcd for $\text{C}_{19}\text{H}_{32}\text{O}_5$: C, 67.03; H, 9.47. Found: C, 68.88; H, 9.39. ^1H NMR: δ 0.90 (t, 3H, $J = 7.3$ Hz, $\text{CH}_3\text{CH}_2\text{CH}_2-$), 1.28 (t, 6H, $J = 7.1$ Hz, $2 \times \text{OCH}_2\text{CH}_3$), 1.29–1.40 (m, 8H, $4 \times -\text{CH}_2-$), 1.86 (m, 2H, $\text{CH}_3\text{CH}_2\text{CH}_2-$), 2.00 (m, 4H, $-\text{CH}_2-\text{CH}=\text{CH}-\text{CH}_2-$), 3.54 (d, 1H, $J = 15.8$ Hz, $-\text{CH}_2-\text{CO}-$), 3.59 (t, 1H, $J = 7.3$ Hz, $-\text{CO}-\text{CH}-$), 3.61 (d, 1H, $J = 15.8$ Hz, $-\text{CH}_2-\text{CO}-$), 4.19 (q, 4H, $J = 7.1$ Hz, $2 \times \text{OCH}_2\text{CH}_3$), 5.30–5.39 (m, 2H, $-\text{CH}=\text{CH}-$).

Methyl 5-oxo-(Z)-12-hexadecenoate (6). A solution of **5** (14.0 g, 41.1 mmol) in dry 1,2-dimethoxyethane (DME, 15 mL) was added dropwise to a stirred solution of NaH (2.5 g of a 50% mineral oil dispersion, 41.1 mmol) in dry DME (50 mL) at room temperature and stirring was continued for 1 h under argon.³⁷ Ethyl 3-bromopropionate (8.9 g, 49.3 mmol) and finely powdered NaI (1.65 g) were then added to the solution and the mixture was refluxed for 18 h with stirring. The reaction mixture was cooled to room temperature and the solvent was evaporated. The residue was heated with 15% aq solution of NaOH (100 mL) under reflux for 24 h and the reaction mixture was acidified with concd HCl, saturated with NaCl and extracted with ether. The dried solution (MgSO_4) was added into an ethereal solution (300 mL) of freshly prepared diazomethane. After 20 h of being stirred the reaction mixture was concentrated in vacuo and the residue was purified by PMPLC. Chromatography afforded 7.1 g (61%) of the ester **6**. Calcd for $\text{C}_{17}\text{H}_{30}\text{O}_3$: C, 72.30; H, 10.71. Found: C, 72.19; H, 10.51. ^1H NMR: δ 0.90 (t, 3H, $J = 7.4$ Hz, $\text{CH}_3\text{CH}_2\text{CH}_2-$), 1.24–1.40 (m, 8H, $4 \times -\text{CH}_2-$), 1.56 (m, 2H, $\text{CH}_3\text{CH}_2\text{CH}_2-$), 1.89 (m,

2H, $J = 4 \times 7.3$ Hz, $-\text{CH}_2\text{CH}_2\text{CO}_2\text{CH}_3$), 2.00 (m, 4H, $-\text{CH}_2-\text{CH}=\text{CH}-\text{CH}_2-$), 2.34 (t, 2H, $J = 7.3$ Hz, $-\text{CH}_2\text{CO}-$), 2.38 (t, 2H, $J = 7.4$ Hz, $-\text{COCH}_2-$), 2.47 (t, 2H, $J = 7.2$ Hz, $-\text{CH}_2\text{CO}_2\text{CH}_3$), 3.67 (s, 3H, $-\text{CO}_2\text{CH}_3$), 5.31–5.39 (m, 2H, $-\text{CH}=\text{CH}-$).

(Z)-12-Hexadecen-5-olide (2c). To a mixed solution of NaBH_4 (111 mg, 2.94 mmol) and $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ (140 mg, 0.392 mmol) in ethanol (10 mL) was added the 5-oxoester (**6**) dropwise (830 mg, 2.94 mmol) with cooling (0°C) and stirring, and the mixture was stirred at room temperature for 8 h. Then, a 10% aqueous solution of NaOH (13 mL) was added and stirring was continued for 1 h. The mixture was acidified to pH 1–2 with concd HCl, stirred for 1 h at 0°C , then extracted with ether (3×25 mL), dried (MgSO_4) and evaporated to give an yellow oil. This oil was added to a stirred solution of trifluoroacetic acid (50 μL) in anhydrous CH_2Cl_2 (20 mL). After 20 h being refluxed, the reaction mixture was concentrated in vacuo. The further purification by PMPLC gave 0.51 g (94%) of pure (GLC) product **2c**. Calcd for $\text{C}_{16}\text{H}_{28}\text{O}_2$: C, 76.13; H, 11.19. Found: C, 75.91; H, 10.98. ^1H NMR: δ 0.90 (t, 3H, $J = 7.3$ Hz, $\text{CH}_3\text{CH}_2\text{CH}_2-$), 1.28–1.94 (m, 16H, $8 \times -\text{CH}_2-$), 2.01 (m, 4H, $-\text{CH}_2-\text{CH}=\text{CH}-\text{CH}_2-$), 2.44 (ddd, 1H, $J = 6.8/8.8/17.6$ Hz, $>\text{CH}-\text{CH}_2-$), 2.58 (dt, 1H, $J = 6.9/6.9/17.6$ Hz, $>\text{CH}-\text{CH}_2-$), 4.28 (m, 1H, $J = 2.9/5.1/7.8/10.6$ Hz, $>\text{CH}-\text{CH}_2-$), 5.32–5.39 (m, 2H, $-\text{CH}=\text{CH}-$). IR (cm^{-1}): 3001 m [$\nu(\text{C}-\text{H})$, *cis*-double bond], 1736 vs [$\nu(\text{C}=\text{O})$, lactone], 1654 w [$\nu(\text{C}=\text{C})$].

Bioassays

Insects. Males of *C. molesta* originated from the laboratory colony maintained on an semiartificial diet under a 16:8 light:dark regime. Moths were sexed as pupae and males were stored separately from females. Newly emerging adults were collected daily, provided with water and sugar solution absorbed onto cotton wool under the same light and temperature conditions. Males 2–4 days old were used for EAG experiments, 3–4 days old males were used for flight-tunnel observation.

Electroantennography. Two glass Ag/AgCl microelectrodes filled with physiological saline were used for EAG recordings: the ground electrode was placed into the head capsule of an intact male moth and the recording electrode was connected with the distal end of the male antenna, the tip of which had been cut off. Antennal responses were amplified (signal conditioner CyberAmp 320, Axon Instruments), digitized (Metabyte DAS-16 A/D, sample period 250 ms) and analyzed by a PC 486 computer (Stand Alone Acquisition System, Run Technologies).

The main pheromone component of the Oriental fruit moth, *C. molesta*, and its analogues **1b–d** and **2a–c** were dissolved in hexane forming a series of dilutions from 5 ng to 5 μg per μL . Aliquots of 5 μL were pipetted onto a filter paper disc (10 mm i.d., Whatman

no. 2) and each loaded disc was inserted into a Pasteur pipetter after solvent evaporation. The odor cartridges were stored deeply frozen in closed glass vials when not used for experimentation. The cartridges conditioned in laboratory temperatures for at least 1 h were used for stimulation. Stimuli were delivered onto the antennal preparation by air puffs blown through the cartridge outlet of which was positioned at a distance 2.5 cm from the antenna. The stimulus duration was 0.8 s, the air flow rate was 1 L min⁻¹. Between successive stimulations the antennal preparation was blown by a continual stream of clean and humidified air. Intervals used between two successive stimuli ranged from 1 to 20 min, depending on the type and intensity of the stimuli. Typically, 1–4 min were adequate for complete recovery of the EAG at lower doses, while 10–20 min were necessary when doses greater than 10 µg were used. Three EAG replicates were recorded for each serial dilution of each odorant. Recordings were repeated on three male antennae. The main pheromone component **1a** (50 ng) served as a standard to normalize EAG responses from different individuals and to control over viability and constancy of the preparation. Stimulation with the standard both preceded and followed each experimental session. The EAG responses to solvent were subtracted from the overall EAG response. EAGs to test chemicals were then expressed as a percentage of the EAG response to the standard stimulation.

Single sensillum recording. Receptor potentials and nerve impulses were recorded extracellularly from receptor cells associated with the *s. trichodea* using a modified tip-cutting technique.³⁸ A whole animal preparation was used. A male in a disposable pipette tip was fixed in place by small droplets of molten wax, while the head and one antenna were protruded. The antenna was carefully bent dorsally and fixed by wax. The tips of *s. trichodea* were cut by means of two glass microknives (microelectrodes with broken tips, ~30 µm i.d.) mounted in micromanipulators. The recording electrode (10 µm in diameter) slipped over cut *s. trichodea* was filled with receptor lymph saline, the reference electrode, inserted in the head, contained saline approximating the ionic composition of the moth haemolymph.³⁸ Prior to the slipping, the tip of the recording electrode was dipped into heated vaseline to prevent it from drying out. The electrical activity of the receptor cells was recorded similarly as EAG recordings on the same instruments. Receptor potentials (DC recordings) and spike activity (AC recording) were recorded simultaneously by two independent channels of signal conditioner.

Short-range behavior. The effect of analogues on male precopulation behavior was investigated in disposable Petri dishes (10 cm i.d.). The compound investigated was loaded on a filter paper disc (10 mm dia) placed in the center of the dish housing the calling female. After 30 min of equilibration a male was introduced into the dish and its behavior was observed for a 30 min period. Experiments were performed simultane-

ously with six pairs of dishes (one test and one control) in four replicate series. Mating efficiencies of males in the test and control dishes were expressed in the form of confusion coefficients, $CC (\%) = (C_c/N_c - C_e/N_e)100$, where CC is the confusion coefficient, C_c no. of copulations in controls, N_c no. of pairs in controls, C_e no. of copulations in the experimental group and N_e no. of pairs in the experimental group.

Flight-tunnel experiments. The CM males were flown in a 1.86 m long × 0.3 m wide × 0.3 m high plexiglass flight-tunnel. Charcoal filtered and humidified air was pushed through the tunnel by four ventilators. The air velocity was maintained at 0.5 ms⁻¹. The flight-tunnel conditions used were: 22–26 °C, 40–60% relative humidity and 700 lux light intensity.

In a preliminary series of experiments male reactions to the calling female to **1a** alone and to the three-component pheromone blend (**1a** 90%, *E*8-12:Ac 6%, *Z*8-12:OH 4%) were determined. Males were allowed to respond to 1, 10 and 100 ng of pheromone blend to determine which odor source is comparable with the calling female. The pheromone blend (10 ng) loaded on a filter paper disc (10 mm dia) was fully comparable with the female and therefore was used as a standard in all flight-tunnel experiment.

Using pheromone analogues, two different types of observation were performed. Firstly, male reactions to the pheromone standard (10 ng) masked by 100 ng of the respective analogue were observed to see if the analogue has an ability to modify the male orientation to odor source. Secondly, to determine if the analogue can substitute the main pheromone component **1a**, males were observed while responding to the odor source in which **1a** was replaced by an appropriate amount of the analogue.

The experiments were performed from 13 to 15 h after the beginning of scotophase. Virgin males (3–4 days old) were placed individually into clean glass tubes (release cages, 10 cm long, 4 cm i.d.) 15 min prior to each session. After 15 min acclimatization period males were released from the central part of the tunnel into an odor plume which was created by pinning the filter paper disc (10 mm dia) loaded with odor onto the holder placed centrally near the upwind end. The filter paper disc created turbulence and so structured the plume (its parameters and orientation was checked using TiCl₄ prior to and after each flight session). Each male was tested once and then discarded. Due to the relatively rapid release rates of volatile chemicals from filter paper sources, only five males were tested for each filter paper source. In six replicate series, altogether 30 males were flown for each treatment. To assure a convenient state of the males, an additional five individuals were tested on the three-component pheromone blend after each day's session.

Male behavior was classified into four categories: (i) activation (walking and wing fanning), (ii) take off, (iii)

oriented flight and (iv) touching the odor source, landing and copulation attempts. The total time of observation was either 2 min if the male did not take off or it lasted until its landing.

Statistical analysis. The data were subjected to statistical analyses utilizing the StatgraphicTM-Plus software package (Manugistic, Rockville, Maryland, U.S.A.). Student's t-test ($\alpha = 0.05$) was used to compare mean responses for differences ($H_0: m_1 = m_2$).

Minimum energy calculations (MEC). MEC were performed for ethyl acetate, ethyl chloroformate, butan-3-olide and pentan-4-olide. These compounds represented those parts of the molecule that were altered. The aliphatic unsaturated chain of the molecules remained constant for all analogues, and was not expected to affect the total conformation of each molecule differently. The PC software used was HyperChemTM for Windows (Autodesk, Sausalito, California, U.S.A.). Energy minimization operations were run until the energy gradient for each molecule was less than 0.1 kJ mol⁻¹.

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