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Dialkyl Phosphorofluoridates and Alkyl Methylphosphonofluoridates as Disruptants of Moth Sex Pheromone-Mediated Behavior

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ABSTRACT

A series of dialkyl phosphorofluoridates and alkyl methylphosphonofluoridates in which one or two of the alkyl moieties were long-chain alkyl groups were synthesized and examined for their ability to disrupt moth sex pheromone-mediated behavior of the oriental fruit moth, Grapholita molesta. *Disruption of pheromone-mediated behavior was found only for those compounds containing a* (Z)*-8-dodecenyl moiety, i.e. the alkyl substituent of the principal pheromone component* ((Z)*-8-dodecenyl acetate) of the oriental fruit moth. Di-*((Z)*-8-dodecenyl) phosphorofluoridate was nearly as disruptive as* (Z)*-8-dodecenyl acetate.*

1 INTRODUCTION

Male moths locate pheromone-emitting females or synthetic pheromone sources using programs of in-flight upwind steering (optomotor anemotaxis) and counterturning.[1] Both programs are switched on by the correct species-specific pheromone blend; thus, successful orientation and mating depends on initial olfactory events occurring at the antennal level. The crucial events involved in pheromone olfaction include adsorption of the airborne pheromone onto the antennal surface, followed by diffusion to a receptor site with subsequent binding and activation of the receptor, either by directly opening ion channels in the membrane or by activating a second messenger, causing a change in membrane conductance. Recent evidence[2–5] indicates that the intermittancy of the pheromone

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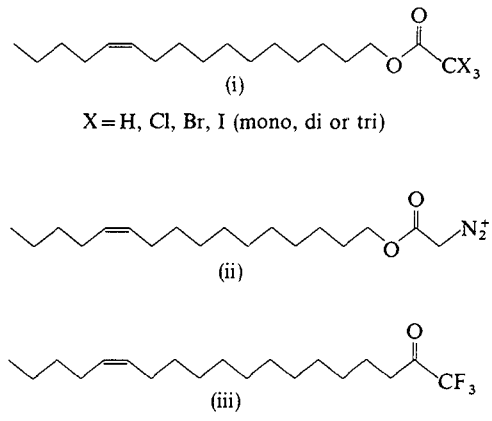


Fig. 1. Potential competitive inhibitors of antennal esterases.

signal in a natural plume that has a fine, filamentous structure is required for the insect to exhibit the sustained upwind flight necessary for locating a pheromone source. Further evidence at the single antennal neuron level indicates that the inability of the antenna to rapidly inactivate the pheromone molecules before the next filament arrives (usually in one second or less) contributes to adaptation of the neurons, which is also correlated with the cessation of sustained upwind flight.[3]

Inactivation is believed to involve, among other things, the chemical alteration of the pheromone to an inactive form. In those cases where the pheromone is a carboxylic ester, inactivation is attributed to rapid hydrolytic degradation, mediated by a carboxylesterase, to the inactive alcohol and acid. Inhibition of the carboxylesterase should result in an accumulation of the pheromone at the receptor sites, resulting in saturation of the sites and adaptation of the antennal neurons. The inability of the cells to disadapt and fire with subsequent exposure to newly-arriving pheromone would turn off at least the anemotactic program involved in source location,[6] and thus prevent males from finding females for mating.

Recently, mono-, di- and tri-halogenated acetates Fig. 1(i), diazoacetate (ii) and trifluoromethyl ketone (iii) analogs of Z11-16:Ac, pheromone of *Plutella xylostella*, were studied as competitive inhibitors of antennal esterases.[7] The fluoro compounds were more inhibitory to esterases, but were electrophysiologically less active than the natural pheromone, and thus were not investigated further. We hypothesized that pheromone carboxylesterase could be inhibited selectively by organophosphorus esters. The general esterase-inhibiting properties of certain phosphate and phosphonate esters are well-known.[8] We have synthesized a series of phosphorofluoridate and phosphonofluoridate esters containing one or two long-chain alkyl groups. Some of these compounds were active in preventing subsequent response to pheromone, and in addition, had pheromone-like activities on males.

2 METHODS AND MATERIALS

2.1 Synthesis

The compounds were routinely checked for their homogeneity on silica gel-G TLC plates. [^{1}H] and [^{13}C]NMR spectra were recorded on a GE-QE 300 instrument. [^{31}P] and [^{19}F]NMR were recorded on a Nicolet NT-300 instrument using phosphoric acid and 1% hexafluorobenzene in deuterobenzene as external reference, respectively. Mass spectra were recorded on a VG-ZAB 2FHF high-resolution mass spectrometer. Molecular formulae were confirmed by exact mass determination in the chemical ionization mode using PFK as a reference material.

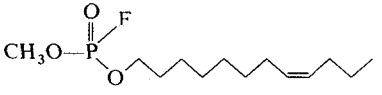
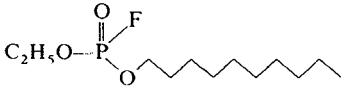
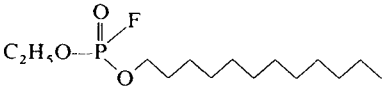
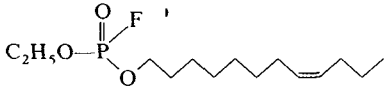
The following methods are illustrative of the procedure employed to prepare the various compounds described in Table 1. Methyl phosphorodichloridate, ethyl phosphorodichloridate, phosphorus oxychloride, methylphosphonic dichloride, decanol, dodecanol and tetradecanol were purchased from Aldrich Chemical Co. (*Z*)-8-dodecenol and (*E*)-8-dodecenol were purchased from Bedoukian Research, Inc. Isopropyl phosphorodichloridate was prepared according to a reported method.[9] The preparation of ethyl dodecyl phosphorofluoridate (**III**) provides a typical example for the synthesis of the compounds in Table 1.

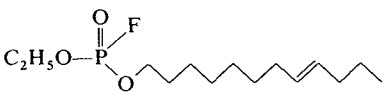
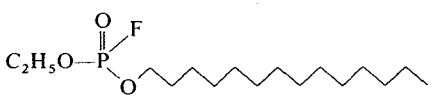
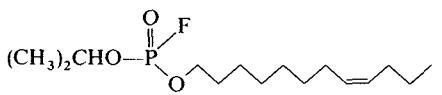
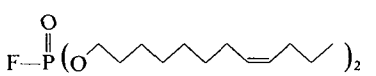
*2.1.1 Ethyl dodecyl phosphorofluoridate (**III**)*

A solution of dodecanol (1·86 g, 10 mmol) and pyridine (0·79 g, 10 mmol) in 25 ml dry benzene was added dropwise to the stirred solution of ethyl phosphorodichloridate (1·62 g, 10 mmol) in 25 ml dry benzene at 0–5°C over 4 h. The reaction mixture was allowed to come to room temperature, the solid separated by filtration, and the solvent evaporated to dryness to give ethyl dodecyl phosphorochloridate in 75% yield along with ethyl didodecyl phosphate as a minor product. Because of the unstable nature of the chloridate, the mixture was not resolved at this stage, and was used as such for the next step. A mixture of the crude ethyl dodecyl phosphorochloridate (0·62 g, 2 mmol) and potassium fluoride (0·2 g, 3·4 mmol) in benzene (20 ml) was refluxed overnight under vigorous stirring. The reaction mixture was filtered and the solvent was removed under reduced pressure. The fluoridate, **III**, and ethyl didodecyl phosphate were resolved by column chromatography over silica gel 32-33 (40 micron) using hexane+ethyl acetate (1+1 by volume) as the solvent system. The final product was tested for homogeneity by silica gel G TLC, [^{1}H], [^{31}P], [^{19}F]NMR, and high resolution mass spectrometry.

In a similar manner, compounds **I**, **II**, **IV–VII**, **X** and **XI** were prepared by reacting the alkanols with methyl, ethyl and isopropyl phosphorochloridates, and methylphosphonic dichloride. Compound **VIII** [di-((*Z*)-8-dodecenyl) phosphorofluoridate] was prepared similarly by starting with phosphorus oxychloride and two equivalents of (*Z*)-8-dodecenol. Compound **IX** [di-((*Z*)-8-dodecenyl) methylphosphonate] was prepared by reacting methylphosphonic dichloride with two equivalents of (*Z*)-8-dodecenol in the presence of excess pyridine in benzene solvent.

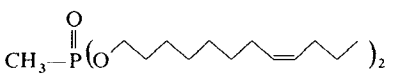
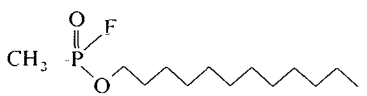
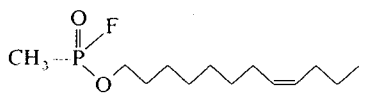
TABLE 1
Physical and Spectroscopic Data of Substituted Phosphates and Phosphonates

Compound No.	*Structure*	*[^1H]NMR (ppm)*	*[^{31}P]NMR (ppm)*	*Mass m/z MH^+*
I		0·82 (t, 3H, CH_3, J=7Hz); 1·25 [m, 10H, $(CH_2)_4$ & CH_2—CH_3]; 1·65 (p, 2H, OCH_2—CH_2, J=7Hz); 1·95 (m, 4H, CH_2—CH=CH—CH_2); 3·84 (d, 3H, OCH_3, J=12Hz); 4·12 (m, 2H, OCH_2, J_{H-H}=7 & J_{P-H}=12Hz); 5·30 (m, 2H, CH=CH)	−7·8(d) J_{P-F}=979Hz	281
II		0·81 (t, 3H, CH_3, J=7Hz); 1·21 [m, 14H, $(CH_2)_7$]; 1·34 (t, 3H, OCH_2—CH_3, J=7Hz); 1·65 (p, 2H, OCH_2—CH_2, J=7Hz); 4·12 (m, 2H, OCH_2—CH_2, J_{H-H}=7 & J_{P-H}=12Hz); 4·19 (m, 2H, OCH_2—CH_3, J_{H-H}=7 & J_{P-H}=12Hz)	−8·9(d) J_{P-F}=979Hz	269
III		0·81 (t, 3H, CH_3, J=7Hz); 1·20 [m, 18H, $(CH_2)_9$—]; 1·33 (t, 3H, OCH_2—CH_3, J=7Hz); 1·65 (p, 2H, OCH_2—CH_2, J=7Hz); 4·10 (m, 2H, OCH_2—CH_2, J_{H-H}=7 & J_{P-H}=12Hz); 4·18 (m, 2H, OCH_2—CH_3, J_{H-H}=7 & J_{P-H}=12Hz)	−8·9(d) J_{P-F}=979Hz	297
IV		0·84 (t, 3H, CH_3, J=7Hz); 1·25 [m, 10H, $(CH_2)_4$ & CH_2—CH_3]; 1·34 (t, 3H, OCH_2—CH_3, J=7Hz); 1·67 (p, 2H, OCH_2—CH_2—, J=7Hz); 1·94 (m, 4H, CH_2—C=C—CH_2—); 4·12 (m, 2H, OCH_2—CH_2, J_{H-H}=7 & J_{P-H}=12Hz); 4·20 (m, 2H, OCH_2—CH_3, J_{H-H}=7 & J_{P-H}=12Hz); 5·29 (m, 2H, CH=CH)	−8·8(d) J_{P-F}=979Hz	295

V 	0·83 (t, 3H, CH_3, J = 7Hz); 1·23 [m, 10H, $(CH_2)_4$ & CH_2—CH_3]; 1·32 (t, 3H, OCH_2—CH_3, J = 7Hz); 1·65 (p, 2H, OCH_2—CH_2, J = 7Hz); 1·94 (m, 4H, CH_2—C=C—CH_2); 4·11 (m, 2H, OCH_2—CH_2, J_{H-H} = 7 & J_{P-H} = 12Hz); 4·19 (m, 2H, OCH_2—CH_3, J_{H-H} = 7 & J_{P-H} = 12Hz); 5·31 (m, 2H, CH=CH)	−8·8(d) J_{P-F} = 979Hz	295
VI 	0·82 (t, 3H, CH_3, J = 7Hz); 1·21 [m, 22H, $(CH_2)_{11}$]; 1·34 (t, 3H, OCH_2—CH_3, J = 7Hz); 1·65 (p, 2H, OCH_2—CH_2—, J = 7Hz); 4·11 (m, 2H, OCH_2—CH_2, J_{H-H} = 7 & J_{P-H} = 12Hz); 4·18 (m, 2H, OCH_2—CH_3, J_{H-H} = 7 & J_{P-H} = 12Hz)	−8·7(d) J_{P-F} = 979Hz	325
VII 	0·80 (t, 3H, CH_3, J = 7Hz); 1·22 [m, 10H, $(CH_2)_4$ & CH_2—CH_3]; 1·32 [d, 6H, 2 × CH_3, J = 7Hz); 1·64 (p, 2H, OCH_2—CH_2, J = 7Hz); 1·94 (m, 4H, CH_2—C=C—CH_2); 4·08 (m, 2H, (CH_2, J_{H-H} = 7 & J_{P-H} = 12Hz); 4·72 (m, 1H, OCH, J_{H-H} = 7 & J_{P-H} = 12Hz); 5·30 (m, 2H, CH=CH)	−9·1(d) J_{P-F} = 977Hz	309
VIII 	0·81 (t, 6H, 2 × CH_3, J = 7Hz); 1·24 [m, 20H, 2 × $(CH_2)_4$ & 2 × CH_2—CH_3]; 1·62 (p, 4H, 2 × OCH_2—CH_2, J = 7Hz); 1·90 (m, 8H, 2 × CH_2—CH=CH—CH_2); 4·06 (m, 4H, 2 × OCH_2, J_{H-H} = 7 & J_{P-H} = 11Hz); 5·25 (m, 4H, 2 × CH=CH)	−8·6(d) J_{P-F} = 977Hz	433

(continued)

TABLE 1—*contd.*

Compound No.	*Structure*	*[^1H]NMR (ppm)*	*[^{31}P]NMR (ppm)*	*Mass m/z MH^+*
IX		0·81 (t, 6H, 2 × CH_3, J = 7Hz); 1·24 [m, 20H, 2 × $(CH_2)_4$ & 2 × CH_2—CH_3]; 1·64 (p, 4H, 2 × OCH_2—CH_2, J = 7Hz); 1·88 (d, 3H, P—CH_3, J = 18Hz); 1·95 (m, 8H, 2 × CH_2—C=C—CH_2); 3·95 (m, 4H, 2 × OCH_2, J_{H-H} = 7 & J_{P-H} = 12Hz); 5·25 (m, 4H, 2 × CH=CH)	30·53	429
X		0·86 (t, 3H, CH_3, J = 7Hz); 1·26 [m, 18H, $(CH_2)_9$]; 1·60 (dd, 3H, P—CH_3, J_{P-H} = 18 & J_{F-H} = 6Hz); 1·65 (p, 2H, OCH_2—CH_2, J = 7Hz); 4·10 (m, 2H, OCH_2, J_{H-H} = 7 & J_{P-H} = 12Hz)	29·67(d) J = 1049Hz	267
XI		0·82 (t, 3H, CH_3, J = 7Hz); 1·25 [m, 10H, $(CH_2)_4$ & CH_2—CH_3]; 1·57 (dd, 3H, P—CH_3, J_{P-H} = 18 & J_{F-H} = 6Hz); 1·64 (p, 2H, OCH_2—CH_2, J = 7Hz); 1·94 (m, 4H, CH_2—C=C—CH_2); 4·12 (m, 2H, OCH_2, J_{H-H} = 7 & J_{P-H} = 12Hz); 5·28 (m, 2H, CH=CH)	29·61(d) J = 1049Hz	265

2.2 Insect and mammalian bioassay

The toxicity of the compounds was obtained by normal methods[10] against a susceptible strain (NAIDM) of female housefly (*Musca domestica*). The compounds dissolved in acetone (1 μl) were applied directly to the notum of the fly and mortality was ascertained 24 h after treatment.

Mouse toxicity was determined orally on female Swiss white mice (25–30 g) obtained from Simonsen Laboratories, Inc.; corn oil was used as the carrier according to usual procedure.[11] The test compounds were dissolved in corn oil and 100 μl of the solution was introduced orally via a syringe equipped with a small animal feeding probe. Mortality was evaluated 48 h after treatment.

2.3 Behavioral bioassay

Approximately 25 oriental fruit moth (*Grapholita molesta*) (Busck) males (3–5 days old) were placed in a 1000-ml pyrex beaker and covered with aluminum foil folded over the top as a lid. A circle of filter paper (5·5 cm Whatman #1) was impregnated with either 10, 100, or 1000 μg of test compound and then placed inside the beaker for 5 min. The test sample at 1000 μg was deposited neat (1 μl), the others as solutions in hexane. After a 5-min exposure, moths were transferred to a wind tunnel for testing their responsiveness to natural sex pheromone.

The 3-m wind tunnel used is based on the design of Miller and Roelofs[12] and is described in detail by Kuenen and Baker.[13] Wind tunnel conditions were: 25($\pm$2)°C, 150 lux lighting from overhead fluorescent bulbs, and 0·5 m sec^{-1} wind speed. Moths were tested during their two-hour maximal pheromone response period, starting one hour prior to scotophase. The natural sex pheromone was impregnated onto the large end of a red rubber septum (5 mm × 9 mm, A. H. Thomas Co.) and consisted of a blend of 10 μg (*Z*)-8-dodecenyl acetate (*Z*8-12:Ac) with 0·6 μg (*E*)-8-dodecenyl acetate (*E*8-12:Ac) and 0·3 μg (*Z*)-8-dodecenyl alcohol (*Z*8-12:OH). The septum was placed on a 15-cm^3 metal platform 15 cm above the tunnel floor at the upwind end where the fan-generated wind blew the odor downwind and out of an exhaust tube at the back of the tunnel.

When first exposed to pheromone, a male *G. molesta* typically fans his wings while walking in circles. The moth then takes flight, 'locks onto' the pheromone plume, and flies in a zigzag pattern upwind towards the pheromone source. A male approaching the source alights nearby, walks 1–5 cm toward the source, turns 180° and extrudes his hairpencils. Occasionally, a male lands directly on the rubber septum.

To initiate a test, a male moth was transferred via a glass vial from the exposure beaker to a screen cone. The cone was then placed on a ringstand situated in the pheromone plume, 2·5 m downwind of the pheromone source. A male was considered successful only if he flew upwind in the plume and hairpencilled after locating the source on the platform, or if he landed on the source. All other moths were considered unsuccessful in locating the source. Moths not leaving the screen cone by 60 sec were forced to do so by vigorously jarring the cone and dislodging the males into the air. Subsequent behavior was recorded as to whether the moth could fly normally; this was one measure of possible deleterious effects of the

pheromone analog. Ten moths were released to each analog dilution; the most concentrated solution was tested first, and no male was used more than once. Typically, testing the ten males in a set took 15 min.

Control moths were treated similarly except that a non-impregnated filter paper circle was placed in the exposure beaker. Four untreated moths were released in the natural pheromone plume before testing of moths exposed to each of the three treatments and after the final treatment in a test series, for a total of 16 males.

Several of the analogs caused males in the exposure beakers to wingfan. Since this behavior is a reliable indicator of upwind flight,[14] a wingfanning bioassay was run to document differential reaction to the analogs. All males were tested during the same two-hour period mentioned above. Males were transferred into individual open-ended glass tubes (10 mm i.d. × 75 mm) with ends covered by aluminum screen. Ten such tubes were placed within one end of a 77-mm i.d. × 610-mm glass tube. The large tube was positioned near a fume hood with the door adjusted to generate an air flow of *c.* 0·5 m sec^{-1} directed toward moths at the downwind end. After 5 min acclimatization, a filter paper impregnated with chemical was held at the upwind end of the large glass tube. The number of males wingfanning just prior to introduction of the analog into the airstream, and at 15-sec intervals over the 2-min exposure were recorded. Ten moths were observed for each dilution of each analog.

3 RESULTS AND DISCUSSION

3.1 Chemistry

Reaction of the alkyl phosphorodichloridates and methylphosphonic dichloride with the various alkanols in the presence of pyridine at 0–5°C gave the corresponding chloridates, along with the formation of minor disubstitution products. Low temperature and slow addition of the alkanol solution helped to avoid the formation of the latter. Phosphorus oxychloride on reaction with two equivalents of (*Z*)-8-dodecenol under similar reaction conditions gave the di-dodecenyl phosphorochloridate. Spectral data for these compounds were found to be in accordance to their structures.

The chloridate intermediates were converted to the corresponding fluoridates by treatment with potassium fluoride. [^{1}H]NMR spectra of the phosphorofluoridates did not show any significant changes from those of the phosphorochloridates except for the OCH_2 protons of **VIII** [di-(*Z*)-8-dodecenyl phosphorofluoridate] which were shifted 0·19 ppm up field from the OCH_2 protons of the corresponding phosphorochloridates. The [^{13}C]NMR of the phosphorochloridate and phosphorofluoridates were virtually identical. The position of the peaks of various carbon atoms of **III** were assigned as: C-12, 14·07; OCH_2—CH_3, 15·93 and 16·02; C-11, 22·65; C-3, 25·18; C-4 to C-9, 28·99–29·58; C-2, 30·02 and 30·11; C-10, 31·88; OCH_2—CH_3, 65·57 and 65·65; and C-1, 69·57 and 69·66 ppm. Interestingly, P—CH_3 protons of **X** and **XI** showed 0·3 ppm up-field shift as double doublet or multiplet, respectively, with P-H = 18 and F-H = 6 Hz coupling values. Chemical shifts of the fluoridates **I–VII** and **VIII** in [^{31}P]NMR ranged

TABLE 2
Properties of Fluoridates

Compound No.	[^{19}F]*Chemical shift (ppm)*	J_{P-F}*(Hz)*	LD_{50} *(μg g^{-1} body weight)*
I	79·17	978	211·7
II	81·3 (d)	977	80·5
III	81·6 (d)	977	27·0
IV	81·9 (d)	977	68·5
V	81·3	977	—
VI	81·6 (d)	977	93·2
VII	83·3 (d)	977	315·4
X	103·5 (dq)	J_{PF} = 1048 & J_{FH} = 6Hz	104·0
XI	103·6 (dq)	J_{PF} = 1048 & J_{FH} = 6Hz	162·7

from −7·8 to −9·1 as doublets with J_{P-F} = 979 Hz, while the phosphonofluoridates **X** and **XI** showed their positions at 29·67 and 29·61 as doublets with J_{P-F} = 1049 Hz, which correspond to literature values.[15] [^{19}F]NMR of the phosphorofluoridates **I–VI** and **VIII** showed their absorptions at 81·0–81·9 ppm, and **VII** was shifted down field to 83·3 ppm. The P-F coupling values (977 Hz) were in accordance with the values observed in [^{31}P]NMR (Table 2). The difference of 1–2 Hz P-F coupling values observed in [^{31}P] and [^{19}F]NMR spectra arose from digital recording difference and experimental errors. Phosphonofluoridates **X** and **XI** showed a double doublet in [^{19}F]NMR at 103·5–103·6 ppm with J_{P-F} = 1048 and J_{F-H} = 6 Hz, corresponding to the values observed in [^{31}P] and [^{1}H]NMR spectra of these compounds. The molecular ion peaks observed in the mass spectra of all the compounds correspond to their calculated molecular weights, and the molecular formulae were confirmed by exact mass determinations.

3.2 Biological activity

The fluoridates were tested for their toxicity against houseflies, and were weakly active (Table 2). Compound **VIII** with two (*Z*)-8-dodecenyl chains was inactive. The activity was compared with that of paraoxon (LD_{50} 0·5 μg g^{-1} body weight). Mammalian toxicity was determined only for the compounds **I**, **IV**, **VIII** and **XI** at a dose of 100 mg kg^{-1} body weight against mice. None of the compounds was found toxic at this dose. Compound **VIII**, which was found most effective in the moth behavioral bioassay, was not acutely toxic to mice at 300 mg kg^{-1} body weight.

Structures of the compounds tested for disrupting *G. molesta* pheromone source location are given in Table 1. In preliminary behavioral bioassays, some of the compounds were significantly active against *G. molesta* (Table 3).

When males were pre-exposed to some of the phosphorofluoridate esters (in particular, **IV** and **VIII**), the number of moths subsequently responding to a plume of the natural pheromone blend by flying upwind and locating the source was reduced significantly. This effect is similar to that of pre-exposure to the major sex pheromone component (*Z*)-8-dodecenyl acetate. It is known[16] that

TABLE 3
Percentage of Moths Flying Upwind and Locating a 3-Component Pheromone Source Following Pre-exposure to the Indicated Compounds

Pre-exposure compound	*Behavior*[a]	*Moths flying upwind (%)*				
		Pre-exposure dosage			*(No pre-exposure)*	
		1000 μg (n=10)	*100 μg (n=10)*	*10 μg (n=10)*	*Control (n=16)*	X^2
I	UpW	60	50	60	75	1·79
	S	60	50	50	75	1·79
II	UpW	60	50	70	56	1·06
	S	60	50	70	56	1·06
III	UpW	60	80	70	50	2·80
	S	70	70	60	50	1·50
IV	UpW	10	20	20	63	10·28[b]
	S	0	20	20	63	13·17[c]
V	UpW	40	40	90	82	10·13[b]
	S	40	40	80	82	8·06[b]
VI	UpW	80	60	80	63	1·74
	S	80	60	80	63	1·74
VII	UpW	60	60	70	88	3·31
	S	60	60	70	88	3·31
VIII	UpW	0	10	10	69	20·16[c]
	S	0	0	10	69	23·50[c]
IX	UpW	80	90	90	75	0·75
	S	80	90	90	75	0·75
X	UpW	70	80	40	75	4·59
	S	70	80	40	75	4·59
XI	UpW	40	80	60	69	3·78
	S	40	70	50	69	2·94
*Z*8-12:Ac (positive control)	UpW	0	0	0	63	23·86[d]
	S	0	0	0	63	23·86[d]

[a] UpW—Upwind flight within the pheromone plume; S—After UpW, male moth located the source.
[b] X^2 significant at $P<0·05$.
[c] X^2 significant at $P<0·01$.
[d] X^2 significant at $P<0·001$.

pre-exposure to natural pheromone causes male moths to habituate and thus become unable to detect and respond to sex pheromone present in the air. Exposure of the males to some of the phosphorofluoridate esters also elicited wingfanning behavior similar to that caused by the natural sex pheromone (Table 4). Notably, **IV** and **VIII**, like the major pheromone component, (*Z*)-8-dodecenyl acetate, caused both wingfanning during pre-exposure and prevention of upwind flight during subsequent exposure of males to a plume of the natural pheromone blend.

As indicated in Tables 3 and 4, highest activity was observed with compounds **IV** and **VIII**, the latter having activity virtually equal to that of the natural pheromone. Both of these compounds were synthesized from *cis*-8-dodecenyl alcohol (*Z*8-12:OH), i.e. the alcohol of the natural pheromone. However, **I**, **VII**

TABLE 4
Responses of Male *G. molesta* When Tested in a Wingfanning Bioassay

	Males wingfanning (%) within 15 s of exposure to compound at indicated dose (μg). n = 10		
	1000	*100*	*10*
I	90	30	10
II	20	0	20
III	20	0	40
IV	100	90	100
V	0	30	0
VI	10	20	20
VII	90	70	80
VIII	100	100	100
IX	90	90	90
X	90	100	100
*Z*8-12:Ac	100	100	100

and **XI**, each also containing the *cis*-8-dodecenyl moiety, were substantially less active than **IV** and **VIII**. Compound **V**, containing the *trans*-8-dodecenyl moiety, was also less active, as were all of the saturated long-chain alkyl esters.

The reason for the high specificity for an ethoxy substituent on the phosphorus (**IV**) compared to the methoxy (**I**), isopropoxy (**VII**) or methyl (**XI**) substituents is not known. It cannot be due to traces of the free alcohol because, although GC analysis revealed 1–3% in some of the analog samples, pre-exposure of males to 100, 10 and 1 mg of *Z*8-12:OH resulted in insignificant reduction of subsequent upwind flight to the pheromone compared to the solvent control. 60%, 67% and 60% of males pre-exposed to the above concentrations of *Z*8-12:OH, respectively, flew upwind and reached the source, compared to 67% for males pre-exposed to the solvent blank ($n = 15$ for all treatments). Likewise, the reduction in response caused by pre-exposure cannot be due to general intoxication of the males because all males that did not respond by flying out of the cage were forcefully dislodged and were capable of normal, coordinated flight.

We wished to test analogs **IV** and **VIII** for their possible interaction with the *Z*8-12:Ac receptor sites on the antennae by upwind flight behavioral assays. Ratios of the natural blend of components that are excessive in the proportion of *Z*8-12:Ac (even highly so) are still extremely effective in luring males all the way to the source.[14,17] On the other hand, blends even slightly deficient (by 15% or more) in the proportion of *Z*8-12:Ac result in nearly complete loss of upwind flight and source contact. Therefore, considering the lower volatility of **IV** and **VIII** compared to *Z*8-12:Ac, as well as their potentially lower affinity to *Z*8-12:Ac receptor sites on the antennae, we created a highly lopsided proportion (10-fold excess) of these two analogs in blends containing the natural minor components *E*8-12:Ac and *Z*8-12:OH. Ten micrograms of the pheromone blend, consisting of 10 parts *cis*-8-dodecenyl acetate (*Z*8-12:Ac), 0·06 parts *trans*-8-dodecenyl acetate (*E*8-12:Ac) and 0·3 parts *cis*-8-dodecenyl alcohol (*Z*8-12:OH) resulted in 75%

success in upwind flight location of the pheromone blend source by males ($n = 10$). Substitution of *Z*8-12:Ac with the same amount of either **IV** or **VIII** in the blend resulted in none of the moths finding the point source of the modified blend, or even initiating upwind flight in the plume. A 10-fold increase in the amount of this blend, or a 10-fold increase of either **IV** or **VIII** in the blend also resulted in no location of the blend source by the moths. These results suggest that **IV** and **VIII** are not acting primarily as mimics of *Z*8-12:Ac and competitively inhibiting receptor sites for this pheromone component or causing neuronal adaptation or habituation. Rather, they appear to be acting by another mechanism, possibly by inactivating the pheromone esterase.

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