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PERFLUORINATED MOTH PHEROMONES
Synthesis and Electrophysiological Activity

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Abstract—Perfluoroalkyl analogs of pheromone constituents were synthesized and responses from male antennal olfactory receptor neurons from three moth species were recorded during stimulation by these analogs. In each analog, the hydrophobic terminus, either a butyl or hexyl substituent on the (Z)-alkenyl chain, was replaced with a perfluorobutyl (Pfb, C_4F_9) or perfluorohexyl (Pfh, C_6F_{13}) moiety. Perfluoroalkyl analogs were more volatile than their hydrocarbon analogs, showing a decrease in gas chromatographic retention time by two to four methylene equivalents (Kovàts retention indices). Specialist neurons of male *Heliothis zea* responded to a 0.02-μg dose of (Z)-11-hexadecenal (Z11-16:Al) and a dose of 200 μg of Pfb-Z11-16:Al with similar spike discharge rates. The HS(a) neurons of *Trichoplusia ni* responded to a dose of 0.02 μg of Z7-14:OAc and a dose of 10 μg of Pfb-Z7-12:Ac with similar discharge rates. The same difference in sensitivity to Pfb-Z7-12:OH and Z7-12:OH was observed for the responses of the HS(b) neuron and for the responses of the NS(a) neurons to Pfb-Z9-14:Ac and Z9-14:Ac. Sensilla of *Diatraea grandiosella* similarly showed 100- to 1000-fold greater sensitivity to Z9-16:Al and Z11-16:Al than to Pfh-Z9-16:Al and Pfb-Z11-16:Al. Thus, replacement of terminal alkyl groups with perfluoroalkyl groups in pheromone components produced biologically active compounds with increased volatility and displaced electrophysiological response profiles.

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Because of the diminished receptor cell sensitivity, we suggest that the binding of the fluorinated analogs to a putative receptor is reduced as a result of less favorable interaction between the hydrophobic protein binding site and the more rigid and more polar perfluoroalkyl moiety.

Key Words—Fluorination, perfluoroalkyl, pheromone analog, Lepidoptera, *Diatraea grandiosella*, *Heliothis zea*, *Trichoplusia ni*, single-cell recording, electrophysiology, receptor binding.

INTRODUCTION

Female moths produce blends of simple saturated and unsaturated fatty alcohols, acetates, and aldehydes that act as sex attractant pheromones for males (Tamaki, 1985). A number of monofluorinated pheromone analogs (Prestwich, 1986; Prestwich et al., 1988; McLean et al., 1989) and selectively fluorinated pheromone mimics (Briggs et al., 1986) have been prepared for previous studies on the molecular basis of pheromone perception and metabolism (Prestwich, 1987a,b; Prestwich and Streinz, 1988). In addition, Camps and coworkers (1986) also have synthesized alkenyl fluorides as pheromone analogs. However, none of these studies has addressed the profound effects on chemical and physical properties of pheromone components that would result from polyfluorination of the alkyl chain. Such polyfluoroalkyl analogs would allow study of the relative importance of ligand hydrophobicity and polarity in receptor binding to antennal transductory proteins (Vogt, 1987; Kaissling, 1986). We describe herein the preparation of analogs of several pheromone constituents in which perfluorobutyl or perfluorohexyl chains are substituted for the normal hydrocarbon termini.

The compounds examined have significance to pheromone-elicited behaviors and selective receptor neuron responses (Mayer and Mankin, 1985) in two noctuid and one pyralid pest species. First, we selected the corn earworm moth *Heliothis zea* (Boddie) (Noctuidae), which has (Z)-11-hexadecenal as a major component in the pheromone blend (Klun et al., 1980). Second, responses from the cabbage looper *Trichoplusia ni* (Hübner) (Noctuidae) were obtained to (Z)-7-dodecenyl acetate and (Z)-9-tetradecenyl acetate, two of the major pheromone components in a redundant blend (Linn et al., 1984). Third, we chose the southwestern corn borer, *Diatraea grandiosella* (Dyer) (Pyralidae), which employs (Z)-11-hexadecenal and (Z)-9-hexadecenal as pheromone components (Hedin et al., 1986). Based on these data, we suggest a model that correlates biological responses with altered physicochemical properties of the pheromone analogs.

METHODS AND MATERIALS

Synthesis of Polyfluoroalkyl Analogs

General. 3-Octyn-1-ol (I), 3-decyn-1-ol (II), and 7-dodecyn-1-ol (III) were purchased from Farchan Labs. Solvents were distilled before use. Anhydrous tetrahydrofuran (THF) and diethyl ether were distilled from sodium benzophenone ketyl prior to use. Flash column chromatography was carried out using Woelm silica gel (32–63 μm). Thin-layer chromatography (TLC) was performed using MN Polygram Sil G/UV254 silica gel plates (4 cm × 8 cm × 0.25 mm). The developed TLC plates were visualized by staining with 3% vanillin (w/v) in ethanol containing 0.3% sulfuric acid or with 10% phosphomolybdic acid (w/v) in ethanol. Proton nuclear magnetic resonance ([^{1}H]NMR) spectra were obtained in $CDCl_3$ solution using a QE-300 spectrometer and 0.03% tetramethylsilane (TMS) as an internal standard; chemical shifts (δ) are expressed as parts per million downfield from TMS. [^{19}F]NMR spectra were obtained on a Nicolet NT-300 spectrometer using trichlorofluoromethane ($CFCl_3$) as an internal standard in benzene-d_6; chemical shifts (ϕ) are expressed as parts per million upfield from $CFCl_3$. Mass spectra (MS), as well as high-resolution mass spectra (HRMS) (70 eV, electron impact), were obtained using a Spectros MS 30 spectrometer with a DS 50 data system. Gas chromatography was carried out on a Varian model 3700 GC equipped with a fused silica capillary column (DB-1 or DB-5, 30 m × 0.263 mm, 0.25-μm film thickness). All glassware, syringes, and needles were dried in an oven at 110°C before use. The glassware was assembled hot and cooled under a flow of dry nitrogen. All of the reactions were carried out under a small positive pressure of dry nitrogen.

The synthetic procedures below are illustrative of the methods used (1) to convert commercially available 3-alkyn-1-ols and 7-dodecyn-1-ol to ω-alkyn-1-ols, (2) to effect free-radical addition of perfluorobutyl iodide (PCR Chemicals) or perfluorohexyl iodide (Japan Halon Co.) to the alkene, (3) isolation of pure (*E*)-alkenyl iodides and their conversion to (*Z*)-alkenes, and (4) functional group modifications to produce the perfluoroalkyl-modified analogs of pheromone alcohols, acetates, and aldehydes for three moth species.

9-Decyn-1-ol (V). This was obtained from 3-decyn-1-ol (II) in >90% yield using the acetylene zipper reaction as described by Abrams and Shaw (1987). [^{1}H] NMR δ 6.663 (t, J = 6.2 Hz, H-1), 2.217 (tt, J = 6.8, 2.6 Hz, H-8) 1.950 (t, J = 2.6 Hz, H-10), 1.65 (m, H-2 to H-7).

9-Iodo-11,11,12,12,13,13,14,14,14-nonafluorobutyltetradec-9-en-1-ol (VIII). 9-Decyn-1-ol (V) (462 mg, 3 mmol), *n*-perfluorobutyl iodide (1.56 g, 4.5 mmol), and azobis(isobutyronitrile) (AIBN, 50 mg, 0.3 mmol) were placed

in a heavy-walled glass tube (2.5 cm OD, 1.8 cm ID × 20 cm, with a narrow neck 5 cm from bottom) equipped with a magnetic stir bar. The mixture was frozen (liquid N_2, 77°K), degassed, and thawed under nitrogen atmosphere to eliminate oxygen. This process was repeated again. Then the tube was sealed under vacuum while the contents were still frozen. The mixture was slowly warmed to room temperature and heated to 80°C for 18 hr. After cooling down with liquid nitrogen, the tube was opened. The crude product was purified and the *E* and *Z* isomers were separated by flash column chromatography (hexane–ethyl acetate, 10:1). Fractions with over 98% isomeric purity (GC) were combined; the remaining fractions were pooled, concentrated, and rechromatographed. After two chromatographic separations, two portions were obtained, isomeric pure *E* isomer of Pfb-9-iodo-Δ9–14:OH (VIII) (750 mg, 50% yield) and a mixture of *E* and *Z* isomers of alkenyl iodide VIII (495 mg, 33% yield). A small aliquot was analyzed by GC, which indicated that before chromatography the *E–Z* ratio was 8.7:1. [^{1}H]NMR δ 6.337 (t, J = 14.5 Hz, H-10), 3.620 (t, J = 6.30 Hz, H-1), 2.609 (t, J = 6.55 Hz, H-8), 1.59 (br s, H-2, H-7), 1.30 (br s, H-3 to H-6); [^{19}F]NMR ϕ 79.90 (tt, J = 12.36, 2.70 Hz, F-14), 104.06 (m, 2F), 122.79 (m, 2F) 124.46 (m, 2F).

The other three perfluoroalkyl alkenyl iodides were prepared in an analogous fashion. The yields and *E–Z* ratios were: (1) 11-iodo-Pfb-Δ 11–16:OH (IX), 80% yield, *E/Z* = 9.2; (2) 7-iodo-Pfb-Δ7–12:OH (VII), 84% yield, *E/Z* = 7.3; (3) 9-iodo-Pfh-Δ9–16:OH (X) = 80% yield, *E/Z* = 7.0.

(Z)-11,11,12,12,13,13,14,14,14-Nonafluorotetradec-9-en-1-ol (Pfb-Z9–14:OH) (XII). A solution of *n*-BuLi (1.6 M soln. in hexane, 3 ml) was added to a solution of Pfb-alkenyl iodide VIII (472 mg, 1 mmol) in 10 ml of dry ether at −78°C. The mixture was stirred for 30 min and quenched with precooled (−78°C) methanol (3 ml). After warming to room temperature, the mixture was poured into 20 ml of NH_4Cl solution and extracted with ether (3 × 15 ml). The combined ether extracts were washed with brine (1 × 10 ml), dried ($MgSO_4$), concentrated in vacuo, and chromatographed (hexane–ethyl acetate, 10:1) to yield 300 mg (83%) of Pfb-Z9–14:OH (XII). [^{1}H]NMR δ 6.117 (dtt, $^3J_{H9,H10}$ = 12.0 Hz, $^3J_{H9,H8}$ = 7.8 Hz, $^4J_{H9,F11}$ = 2.4 Hz, H-9), 5.472 (m, H-10), 3.625 (t, 6.1 Hz, H-1), 2.294 (br s, H-8), 1.61 (m, H-2, H-7), 1.30 (br s, H-3 to H-7). [^{19}F]NMR ϕ 80.84 (t, F-14), 105.79 (m, 2F), 123.99 (m, 2F), 125.24 (m, 2F). FT-IR (neat): 3333.2 br, 2930.9, 2858.5, 1663.0, 1353.5, 1235.7, 1133.6, 878.4, 742.7 cm^{-1}.

Pfb-Z9–14:Al (XV). A mixture of Pfb-Z9–14:OH (XII) (38 mg, 0.1 mmol), 4 Å molecule sieves (10 mg), and 4-methylmorpholine *N*-oxide (NMO, 27 mg, 0.15 mmol) in 2 ml of dry methylene chloride was stirred at 25°C. After 10 min, tetrapropylammonium perruthenate (TPAP, 0.5 mg) was added to the mixture. The color of the solution slowly changed from light green to dark brown; TLC showed the reaction was completed after another 10 min. The

mixture was filtered through silica gel, concentrated in vacuo, and purified by flash chromatography (hexane–ethyl acetate, 20:1) to yield 31 mg (81%) of Pfb-Z9–14:Al (XV). [^{1}H]NMR δ 9.772 (t, J= 1.22 Hz, H-1), 6.094 (dtt, $^3J_{H9,H10}$ = 12.10 Hz, $^3J_{H9,H8}$ = 7.69 Hz, $^4J_{H9,F11}$ = 2.30 Hz, H-9), 5.493 (m, H-10), 2.472 (td, J = 7.32, 1.20 Hz, H-2), 2.36 (br s, H-8), 1.61 (m, 1.35 br s, 8H); [^{19}F]NMR was unchanged from that for Pfb-Z9–14:OH.

Pfb-Z7–12:Ac (XVIII). A mixture of Pfb-Z7–12:OH XI (35 mg, 0.1 mmol) (prepared analogously to Pfb-Z9–14:OH), acetic anhydride (36 mg, 0.3 mmol), and 4-dimethylaminopyridine (DMAP, 5 mg) in 2 ml of pyridine was stirred at room temperature for 3 hr. The mixture was diluted with 30 ml of hexane–ethyl acetate (7:3) solution, washed with saturated $CuSO_4$ solution (3 × 1 ml), dried ($MgSO_4$), concentrated in vacuo, and chromatographed (hexane–ethyl acetate, 50:1) to yield 36 mg (92%) of Pfb-Z7–12:Ac (XVIII). [^{1}H]NMR δ 6.127 (dtt, $^3J_{H7,H8}$ = 12.05 H, $^3J_{H7,H6}$ = 7.84 Hz, $^4J_{H7,F9}$ = 2.35 Hz, H-7), 5.522 (m, H-8), 4.050 (t, J = 6.32, H-1), 2.306 (brs, H-6), 2.025 (s, acetate), 1.61 (m, H-2), 1.30 (brs, H-3 and H-5); [^{19}F]NMR, no significant difference from Pfb-Z9–14:OH.

Electrophysiological Techniques

Insects. Adult *H. zea* and *T. ni* were obtained from a colony maintained by the Insect Attractants, Behavior, and Basic Biology Research Laboratory, Gainesville, Florida. Adult *D. grandiosella* males were obtained from a colony maintained by the Crop Science Laboratory, Mississippi State, Mississippi.

H. zea and T. ni. Details of the stimulus delivery device and procedures are described by Grant et al. (1989). The wings and legs of intact specimens were secured intact in a hollowed-out depression of a small Plexiglas plate with molten paraffin–beeswax. Basal and distal ends of the antenna were immobilized between strips of double-stick Scotch tape so that several distal flagellomeres (antennal segments) were accessible to the electrodes and stimuli.

Action potentials (spikes) were recorded by inserting uninsulated sharpened tungsten electrodes at the base of sensilla trichodea located over the lateroventral region of the antenna. The electrical responses were amplified by an AC-coupled Grass P-15D preamplifier and band-pass filtered (3–300 Hz). The impulse activity was monitored on a storage oscilloscope, and the signal was further amplified and led to a Digital PDP 11/23 computer. The digitized spike trains were stored and analyzed according to Mankin et al. (1987). The number of spikes per second was obtained by subtracting the number of spikes in the prestimulus interval from the number in the stimulus interval and dividing by the stimulus duration (3 sec).

The stimuli were delivered from a glass delivery device that maintained a constant flow of synthetic compressed air (Linde, Inc.) purified by passage first

through silica gel, then activated charcoal, and finally through silica gel. The constant airflow isolated the antenna from room air and carried the stimulus from the dispenser. The delivery device was rinsed with acetone and aerated following all stimuli at concentrations that produced a residual sufficient to elicit a response. The output of the stimulus delivery device was converted to concentration based on calibrations in Mayer et al. (1987). Vapor concentrations of the perfluoroalkyl analogs and nonfluorinated stimuli were essentially identical at the same dosage level (see calibration data below).

D. grandiosella. Electroantennogram (EAG) techniques were adapted from earlier methods (Schneider, 1957) and described elsewhere (Dickens, 1979; Hedin et al., 1986). Briefly, two glass microcapillary electrodes filled with physiological saline (Oakley and Schafer, 1978) were inserted into the antenna of an intact animal; the ground electrode was placed into the fourth or fifth antennal flagellomere, and the recording electrode was inserted into the terminal or penultimate antennal segment. Electrical contact was provided by a Ag–AgCl wire placed into the shank of each capillary. Electrical activity was amplified 10× by a Grass P-16 DC preamplifier. EAG waveforms were visualized on a Tektronix 5111 analog storage oscilloscope and recorded using a stripchart recorder. Stimulus duration was 1 sec with an airflow of 1 l/min. Three minutes were allowed between stimuli at lower dosages; 5–7 min were allowed at dosages greater than 1 mg. These times were adequate for complete recovery of the EAG.

The stimuli, Z11–16 : Al, Z9–16 : Al, Pfb-Z11–16 : Al, and Pfb-Z9–16 : Al, were delivered from the lowest to the highest dose. Filter papers (8 mm × 18 mm) were treated with 1-μl aliquots of serial dilutions and were inserted into glass cartridges (80 mm × 5 mm ID) that were oriented toward the preparation from a distance of 1 cm. Responses of three *D. grandiosella* males were recorded for each dose of each pheromone and pheromonal analog. 1-Hexanol (100 μg; 10 μl of 10 μg/μl dissolved in pentane) served as a standard to normalize responses, to ensure viability and constancy of the preparation, and to allow relative comparisons with previously recorded species (Dickens, 1984). Stimulation with the standard both preceded and followed each serial dilution level. No responses were obtained to a solvent control. EAGs were expressed as a percentage of the mean of the two nearest responses to the standard (Dickens, 1981). The threshold response was considered to be the dosage at which the standard error of the mean was not equal to or less than zero (Dickens, 1984).

Single-cell recording techniques are described in detail elsewhere (Dickens, 1979; Dickens and Mori, 1988) and were modified from Boeckh (1962). In brief, microelectrodes used for recordings were constructed from 50.8-μm-diameter tungsten wire electrolytically sharpened to a tip of 1–2 μm. The recording electrode was positioned under optical control (150–200×) by a Leitz micromanipulator near the base of a single sensillum trichodeum. The ground

electrode was implanted in a distal antennal segment. Action potentials were amplified by a Grass P-15 preamplifier and displayed on a Tektronix 5223 digitizing oscilloscope. The signal was recorded on a Teac R51-D data recorder and displayed on a Tektronix 5111 analog oscilloscope during storage on the hard disk of a Dell Corporation 286 microcomputer. Trains of action potentials were counted visually from the oscilloscope screen or analyzed with the aid of the microcomputer utilizing SAPID (Spike Analyses Programs for Insect Data) from Tasteful Software Laboratory, Department of Entomology, University of Alberta, Edmonton, Alberta. The stimulus delivery system was the same as that described for single cell stimulations; stimulus duration was 0.5 sec.

Quantification of Analogs by Gas Chromatography

Detector Response Factors. For Z9–14 : Ac, Pfb-Z9–14 : Ac, and *n*-hexadecane, doses of 1–50 ng were injected into Varian 3700 gas chromatograph equipped with a capillary column (DB-5 0.263 mm ID × 30 m). A plot of the area response (by flame ionization detection) to these compounds vs. the amount injected was linear over this range. The relative area responses are: *n*-hexadecane (1.00), Z9–14 : Ac (0.91), and Pfb-Z9–14 : Ac (0.20). These tests were crucial in calibrating the GC for volatilization assays.

Volatilization of Analog during Preparation of Delivery System. The dispenser device used was that described by Mayer et al. (1984). The Z9–14 : Ac or Pfb-Z9–14 : Ac diluted in 0.5 ml of hexane was pipetted into the assembly. The dispenser was rotated under aeration at 200 ml/min for 30 sec to coat the inside uniformly. Then the dispenser was washed with hexane (2 × 1 ml). The recoveries of both Z9–14 : Ac and Pfb-Z9–14 : Ac at 10-, 50-, and 100-μg dosages were over 95% as determined by GC. This test was used to confirm that there were no gross differences in physical properties for the fluorinated and nonfluorinated compounds that would affect release from the dispenser device.

RESULTS

Synthesis of Polyfluoroalkyl Pheromone Analogs

Starting from commercially available 3-octyn-1-ol (I), 3-decyn-1-ol (II), and 7-dodecyn-1-ol (III), the perfluoroalkyl pheromone analogs XV, XVI, XVII, and XVIII could be synthesized in four steps in 40–45% overall yield (Figure 1). For simplicity, we employ the prefix "Pfb" to indicate the perfluorobutyl modification and "Pfh" to indicate the perfluorohexyl modification.

The synthesis of Pfb-Z9–14 : Al (XV) is described as a representative example. 9-Decyn-1-ol (V) was obtained from 3-decyn-1-ol (II) in 85% yield according to the zipper reaction as described by Abrams and Shaw (1987). The

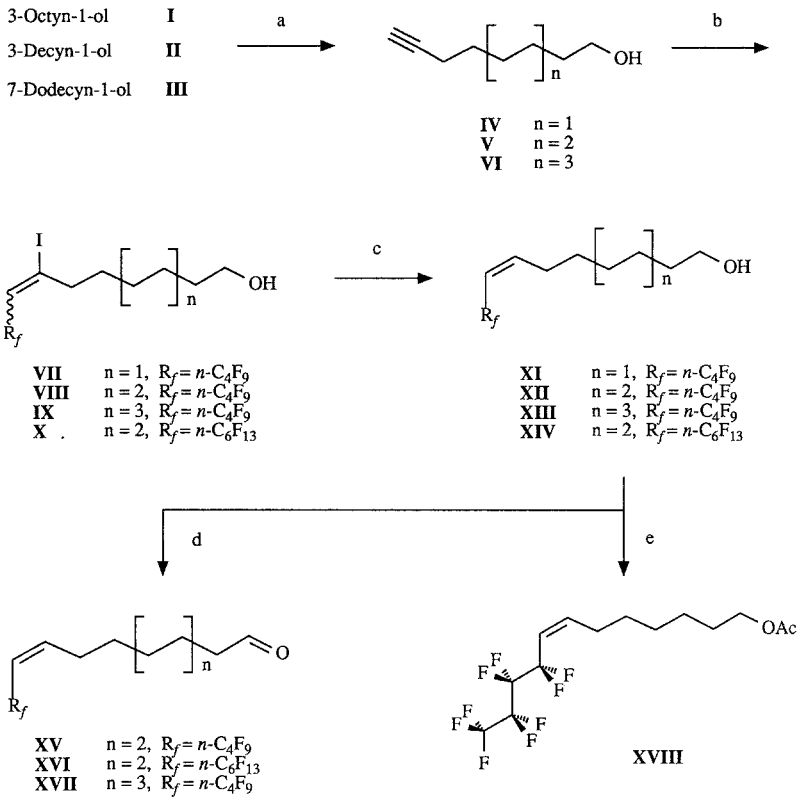

FIG. 1. Synthesis of perfluoroalkyl pheromones. Reagents and conditions: (a) NaH, 1,3-diaminopropane, 80°C, 2 hr, 80–85% yield; (b) n-C_4F_9I or n-$C_6F_{13}I$, azobisisobutyronitrile, 80°C, 18 hr, 80–85%; (c) 1. SiO_2 separation, 2. n-BuLi, THF, −78°C, 0.5 hr, 3. MeOH, −78°C, 75–80%; (d) N-methylmorpholine N-oxide, tetrapropylammonium perruthenate, 4 Å molecular sieves, CH_2Cl_2, 14 min, 85–90%; (e) acetic anhydride, 4-dimethylaminopyridine, pyridine, 45°C, 3 hr, 94%.

ω-alkynol was heated with 1.5 equivalents of perfluorobutyl iodide and catalytic amount of azobis(isobutyronitrile) (AIBN) in a sealed heavy-wall glass tube for 18 hr under nitrogen atmosphere (Fuchikama and Ojima, 1984; Ishihara et al., 1986, Matsubara et al., 1987). The crude alkenyl iodide was isolated as a mixture of E and Z isomers with a ratio of 8 : 7 (Table 1). The E and Z perfluorobutyl vinyl iodides VIII could be separated by flash column chromatography; fractions with over 98% isomeric purity (GC) were combined and concentrated for subsequent reactions. It is important to separate the alkene isomers at this stage, as discussed below.

Deiodination of the (E)-alkenyl iodide VIII with n-butyllithium at −78°C

TABLE 1. KOVÀTS INDICES (GAS CHROMATOGRAPHY RETENTION INDICES)

	X = OH	X = Al	X = Ac
Perfluoroalkyl analogs			
Pfb-Z11-16:X	1744	1558	1744
Pfb-Z9-14:X	1427	1366	1558
Pfb-Z7-12:X	1250	1185	1363
Pfh-Z9-16:X			1646
Natural pheromone components			
Z11-16:X		1891	1982
Z9-14:X		1672	1776
Z7-12:X			1577

followed by quenching with methanol at that temperature afforded the pure *Z* isomer XII with retention of geometry. (Note that the priority rules give iodide a higher priority than alkyl; nonetheless, the alkenyl chain is *cis* in both compounds.) It is important to keep the concentration of the iodide below 0.1 M to provide good yield and a clean reaction, since the solubility of the dianion was a determining factor in this lithium–halogen exchange reaction. Oxidation (TPAP, NMO, CH_2Cl_2) or acetylation (Ac_2O, DMAP, CH_2Cl_2) of the perfluorobutyl alkenol provided the Pfb-Z9-14:Al (XV) or Pfb-Z7-12:Ac (XVIII) [from Pfb-Z7-12:OH (XIV)] in very good yields. It is worth noting that the separation of *E* and *Z* isomers by flash column chromatography was only possible on the perfluorobutyl alkenyl iodides. The *E* and *Z* isomers of all subsequent perfluoroalkyl analogs were inseparable by silica gel chromatography, even using argentation conditions known to separate the corresponding nonfluorinated compounds.

The perfluoroalkyl pheromone analogs are significantly more volatile than their parent compounds, as shown by the lower values for the Kovàts retention indices (Kovàts, 1958) (Table 1). Thus, even though the molecular weights of the perfluorobutyl compounds are 128 mass units higher, the loss of intermolecular hydrophobic interactions renders the molecules less cohesive in the condensed phase. For the aldehydes, adding nine fluorines is the equivalent of shortening the chain by three methylene units!

The concentration of the perfluoro analogs delivered to the antenna is essentially the same as that of the natural pheromone stimulus. Quantification by GC can be deceptive, because the molar response to highly fluorinated molecules is drastically reduced because of the large portions of nonflammable $-CF_2-$linkages. Thus, detector response curves were generated for Pfb-Z9-14:Ac, *n*-hexadecane, and Z9-14:Ac from 1 ng to 50 ng/injection. Relative

to *n*-hexadecane (1.00), these factors were 0.91 for Z9–14:Ac and 0.20 for Pfb-Z9–14:Ac. Next, we determined that >95% of both Pfb-Z9–14:Ac and Z7–14:Ac remained in the delivery device after evaporation of 0.5 ml of hexane solutions containing 10, 50, and 100 μg of each compound.

Electrophysiological Recordings

H. zea. Recordings from neurons within the long, sexually dimorphic sensilla on the proximal 40 or so antennal flagellomeres demonstrated that a 200-μg dose of Pfb-Z11–16:Al elicits about the same number of spikes as a 0.02-μg dose of Z11–16:Al (Figure 2).

T. ni. Three different receptor neurons contained within two different types of sensilla responded to fluorinated analogs of their most effective pheromone stimuli. The HS sensillum has two highly sensitive and selective receptor neurons, one that responds to Z7–12:Ac [HS(a)] and another that responds to Z7–12:OH [HS(b)] (O'Connell et al., 1983). The perfluorinated analogs of each of these compounds elicited a response from the appropriate neuron at a dose of about 10 μg, compared with thresholds of about 0.02 μg of the native compounds (Figure 3). The difference in sensitivity between the perfluorinated analogs and the natural components, consequently, was about the same as the receptor neurons of *H. zea*.

Another highly selective and sensitive receptor neuron is found within a different sensillum on the antenna of the cabbage looper that responds to Z9–14:Ac (Mayer, unpublished). Both Pfb-Z9–14:Ac and Z9–14:Ac elicited a

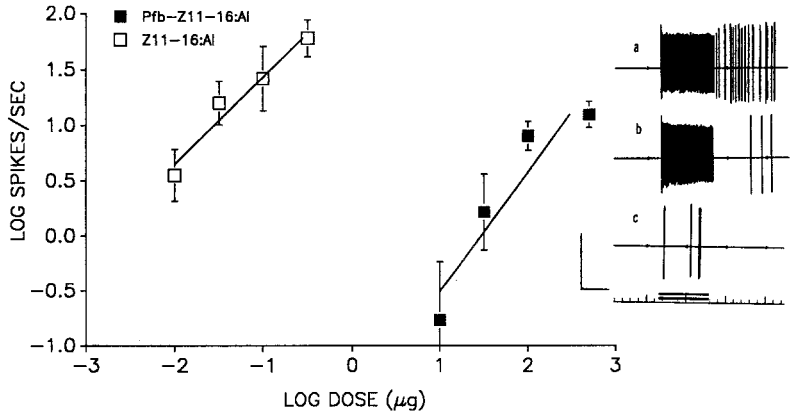

FIG. 2. Averaged responses (±SEM) of six *H. zea* olfactory receptor neurons to Z11–16:Al standards (open squares) and Pfb-Z11–16:Al (closed squares). Inset: response to (a) 1 μg Z11–16:Al; (b) 250 μg Pfb-Z11–16:Al; and (c) 10 μg of Pfb-Z11–16:Al. Calibration: vertical bar = 1 mV; horizontal bar = 1.5 sec.

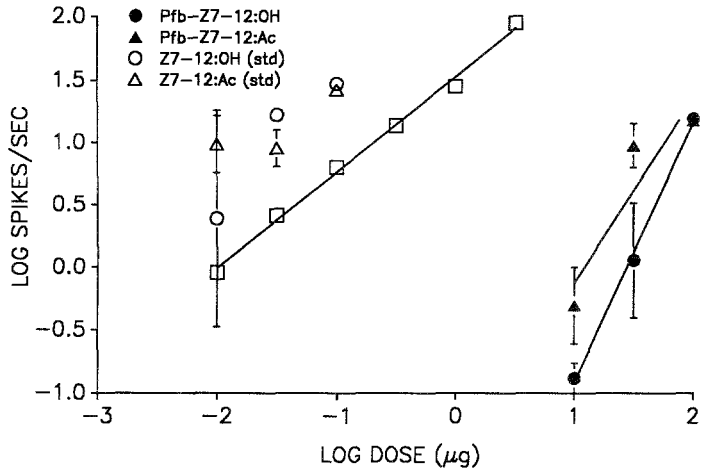

FIG. 3. Averaged responses (±SEM) of five *T. ni* HS(a) neurons to Pfb-Z7-12:Ac (closed triangles) and Z7-12:Ac (open triangles). Responses of the HS(b) olfactory receptor neurons within the same sensilla to Pfb-Z7-12:OH (closed circles) and Z7-12:OH (open circles). A standard response curve from a larger sample of HS(a) neurons is provided for reference (open squares) (Mayer, unpublished).

response from this neuron (Figure 4). The response elicited by a 32-μg dose of the perfluorinated analog was equivalent to a dose of about 0.01 μg of Z9-14:Ac, a difference of about four orders of magnitude.

D. grandiosella. Male antennae responded to serial dilutions of Z9-16:Al

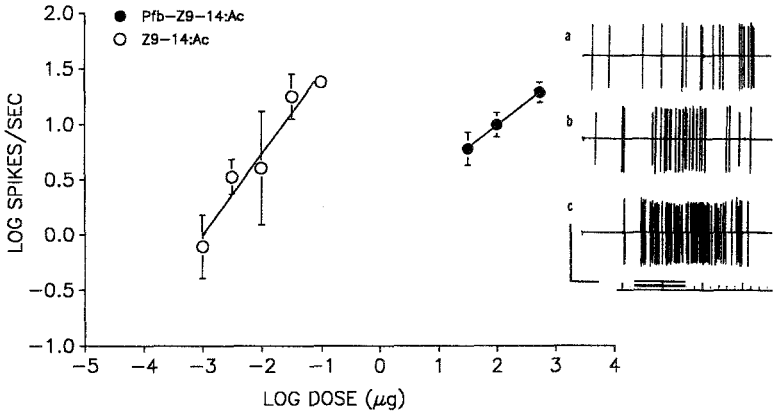

FIG. 4. Averaged responses (±SEM) of six *T. ni* olfactory receptor neurons to Z9-14:Ac (open circles) and Pfb-Z9-14:Ac (closed circles). Inset: responses to (a) 32 μg (b) 100 μg, and (c) 500 μg doses of Pfb-Z9-14:Ac. Calibration: vertical bar = 1 mV; horizontal bar = 1 sec.

and Z11-16:Al, showing thresholds at doses of <0.001 μg with saturation occurring at 1 μg for both compounds (Figure 5A and B). The EAG threshold for both Pfh-Z9-16:Al or Pfb-Z11-16:Al appears to be at doses of approximately 0.01-1 μg. Thus, the thresholds for the perfluoroalkyl analogs are about 100- to 1000-fold higher than the actual pheromone. Above threshold, the slope of the dose-response curve for each Pfb analog is similar to that for the native pheromone components.

Comparison of action potentials recorded from five sensilla trichodea on

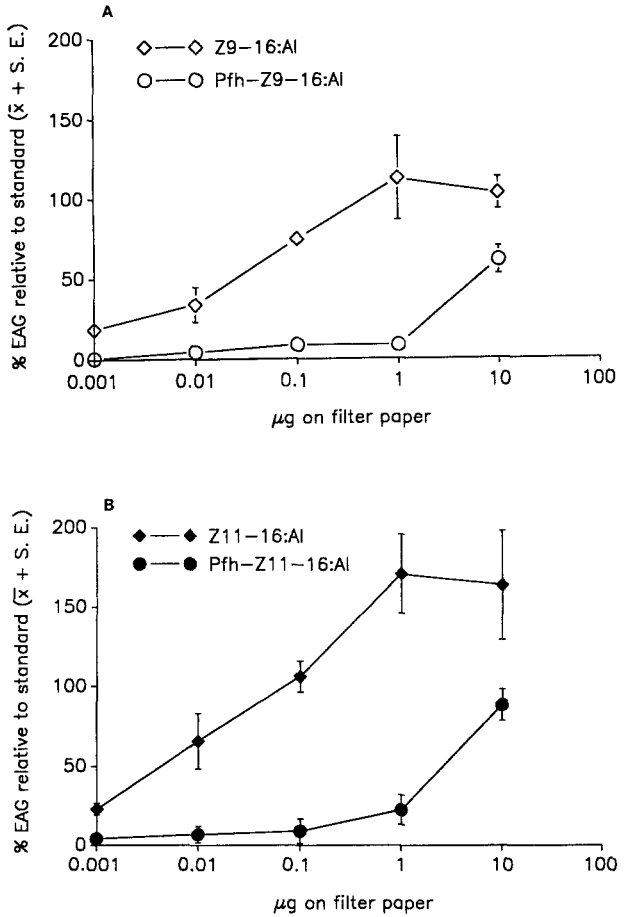

FIG. 5. Dose-response profiles for EAG recordings from *Diatraea grandiosella* male antennae. (A) Relative responses (±SEM) to Z9-16:Al (open diamonds) and Pfh-Z9-16:Al (open circles); (B) relative responses (±SEM) to Z11-16:Al (closed diamonds) and Pfb-Z11-16:Al (closed circles).

the male antenna revealed three receptor neurons responsive to Z9–16 : Al and three neurons responsive to Z11–16 : Al. Two of the neurons responsive to each pheromone component were also activated at a low level by the perfluorobutyl analogs. For example, 1-μg doses of a Z9–16 : Al elicited 80 spikes/initial 400 msec, while a dose of 10 μg of Pfh-Z9–16 : Al elicited 15 spikes/initial 400 msec (spontaneous activity = 3 spikes/400 msec). These results are consistent with the EAG results and indicate the ability of these analogs to stimulate their respective pheromone receptor cell neurons.

DISCUSSION

Volatile signals involved in chemical communication in insects are known to have narrowly defined criteria of molecular structure and physical properties. Among the key molecular properties are chain length and branching, type and location of functionality, degree of unsaturation, alkene geometry, and chirality. The macroscopic properties that affect both emission and reception of a chemical signal include volatility, lipophilicity, dipole moment, and chemical stability. On the molecular level, the introduction of perfluoroalkyl chains into insect pheromones dramatically increases chain bulkiness and reduces chain flexibility and hydrophobicity (Filler and Kobayashi, 1982). At the macroscopic level, the polyfluoroalkyl compounds are more volatile, less lipophilic, have larger dipole moments, and are more stable. Recently, this general class of semifluorinated hydrocarbons was described as "primitive surfactants" (Turberg and Brady, 1988), based on their unusual physical properties and the dual polar–nonpolar nature imparted by perfluorination of one end of a hydrocarbon chain. Stated simply, perfluoroalkyl groups have unique properties that reduce their solubility in both aqueous (hydrophilic) and lipid (hydrophobic) environments.

Bestmann and coworkers (Bestmann et al., 1987; Bestmann and Vostrowsky, 1982; Bestmann, 1986) and others over the last two decades have provided evidence from extensive EAG analyses that demonstrates the importance of alkyl chain length, alkene geometry, alkene position, branching, and functionality in determining the electrophysiological activity of insect pheromones and pheromone analogs. The existence of a chiral recognition site on a macromolecule also was inferred through the demonstration that chiral analogs of an achiral pheromone were perceived differently (Chapman et al., 1978). Energetic profiles of receptor site interactions were discussed in a theoretical sense by Kafka (1976) and by Kafka and Neuwirth (1975), and early efforts to correlate moth sex pheromone activities with molecular conformations were described by Kikuchi (1975). However, major computational advances have been introduced recently by chemical ecologists in the Lund group. The con-

formational energy of pheromone analogs calculated by molecular mechanics can now be correlated with electrophysiological potency (Liljefors et al., 1985, 1987), thus providing experimental evidence supporting a specific macromolecular binding site with spatial recognition for a relatively small set of steric and electronic features.

The responses of pheromone-sensitive receptor neurons in two species of noctuid moths and one pyralid moth to fluorinated pheromone analogs provide a new set of data in support of the importance of the hydrophobicity of the terminal alkyl group in pheromone–receptor protein interactions. In each of the three species examined, the pheromone receptor neurons that are specialized to detect particular pheromone components responded to the homologous perfluorinated molecules, although at elevated concentrations. *H. zea* sensilla that contain receptor neuron(s) responding to Z11–16:Al also were stimulated by the perfluoro analogs. Some other neurons within these same sensilla that responded to Z11–16:Al failed to respond to the Pfb analogs, however. Moreover, the neuron in the HS sensillum of *T. ni* that responds to Z7–12:OH did not respond to the perfluorinated analog of Z7–12:Ac; neither did the neuron recognizing Z7–12:Ac respond to Pfb-Z7–12:OH. Thus, the neuronal responses are consistent with the notion that although perfluorination changes the polarity, hydrophobicity, and conformational rigidity of the pheromone molecule, it does not change it sufficiently to completely alter the overall shape or the dependence on alkene position, alkene geometry, or terminal functionality.

Another feature of the neuronal response to these fluorinated analogs is common to *H. zea* and *T. ni* and, by inference, to *D. grandiosella*. Usually, following the response to high concentrations of the native pheromone components of *T. ni*, there is an elevated poststimulus discharge frequency (Grant et al., 1989). Responses to perfluorinated compounds in both *H. zea* and *T. ni* resulted in a reduced poststimulus discharge by most neurons at any concentration assayed (Figure 2). We interpret this phenomenon in two ways. First, the high concentrations of these compounds required to elicit an increase in spike frequency creates significant demands on their purity. For example, a 0.2% impurity of Z11–16:Al in a dose of over 500 μg means that 1 μg would be in the sample. This amount is sufficient to elicit a significant response by the receptor neurons (Figure 2, inset a). Thus, the lack of a poststimulus discharge (Figure 2, inset b) suggests that the response to the perfluorinated compound is not due to a Z11–16:Al contaminant, which would also be unexpected based on the synthetic scheme.

Second, the diminished poststimulus discharge suggests that although the less hydrophobic perfluorobutyl analogs of Z11–16:Al can stimulate the receptor that recognizes their shape, functionality, and chain length, the reduction in dispersive interaction results in their more rapid dissociation from the receptor. This lack of an increased poststimulus spike frequency also is observed in

response to unphysiologically high concentrations of pheromone components that are poor stimuli of a receptor neuron (Mayer, unpublished). Thus, on the basis that fluorinated analogs act as ligands with poor affinity for hydrophobic regions of pheromone receptors, we have categorized this class of compounds as "nonstick" pheromone analogs. We further speculate that the receptor site may react similarly to other inappropriate pheromonal stimuli at unphysiologically high concentrations.

Two decades of work by Kaissling (1986) led to the hypothesis that long recoveries are due to slow inactivation or slow removal of the stimulant. If this were true for the perfluoroalkyl analogs, then increased removal or inactivation would be required. We prefer the alternative kinetic explanation, i.e., that reduced receptor affinity requires a higher concentration of stimulant to achieve the same level of receptor activation and subsequent signal transduction.

Two very recent precedents that corroborate our observations can be seen in the responses of a moth and a mosquito to fluorinated pheromone analogs. First, the western spruce budworm, *Choristoneura occidentalis*, shows a 14-fold shorter recovery period in EAG responses to the terminally fluorinated analog 14-F-*E*11–14 : Al relative to the recovery period for responses to *E*11–14 : Al (McLean et al., 1989). These researchers suggested increased degradation and transport as the primary explanation for the shortened recovery period. However, both their results and ours seem to provide stronger evidence for the primary effect being the alteration of the hydrophobicity of the terminal methyl group and thus reduction in receptor affinity.

Second, the Pickett group in Rothamsted (Dawson et al., 1990) has independently discovered biological activity in perfluorinated pheromone analogs. In their example, a C_8F_{17} group was substituted for a terminal octyl group in an analog of (−)-(5*R*,6*S*)-6-acetoxyhexadecanolide, the oviposition attractant of the mosquito *Culex quinquefasciatus*. This analog was a potent oviposition stimulant in field tests.

Current biochemical evidence on the molecular mechanism of olfaction in vertebrates (Pace and Lancet, 1987; Snyder et al., 1988) and insects (Vogt, 1987) suggests that pheromones associate with pheromone-specific soluble carrier proteins in the olfactory hair lumen and with membrane-associated receptor proteins on the dendritic surface. For example, in the moth *Antheraea polyphemus*, a male-specific sensory hair membrane protein (69 kDa) and a soluble pheromone binding protein (15 kDa) have been selectively photoaffinity labeled using a physiologically active diazoacetate analog of the pheromone (*E*,*Z*)-6,11-hexadecadienyl acetate (Vogt et al., 1988; Ganjian et al., 1978). That a pheromone analog of similar size and shape, but dramatically different lipid solubility, exhibits pheromonal activity at a reduced level, is most consistent with a key role for a macromolecular receptor-pheromone complex in olfactory transduction.

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