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MOTH RESPONSES TO SELECTIVELY FLUORINATED SEX PHEROMONE ANALOGS

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Abstract—Partially fluorinated analogs of the European corn borer (*Ostrinia nubilalis*) female sex pheromone, 11-tetradecenyl acetate (97:3 *Z*:*E*), having mono- and trifluorosubstitutions at the terminal carbon of the pheromone chain, mimicked the biological activity of the pheromone, while analogs with fluorine at either side of the double bond and a pentafluoro analog were essentially inactive. Comparison of the pheromonal activity of these analogs with the previously reported activity of similarly fluorinated pheromones in five other species of moths revealed an unpredictable relationship between fluorine substitution pattern and pheromone-mimicking activity. Fluorine substitution patterns that rendered pheromonal analogs biologically inactive in the European corn borer had no detrimental influence upon pheromonal activity in other species and the converse was also true. This is evidence that the relative importance of electronic qualities of sites within a pheromone molecule differ from species to species. Furthermore, it indicates that the biochemical components (pheromone receptor proteins, binding proteins, and enzymes) that make up moth olfactory chemosensory systems must also vary structurally from species to species, despite the fact that they are involved in olfactory sensing of compounds having very similar chemical structure.

Key Words—Lepidoptera, Pyralidae, *Ostrinia nubilalis*, 11-tetradecenyl acetate, (*Z*)-14-fluoro-11-tetradecenyl acetate, (*Z*)-14,14,14,-trifluoro-11-tetradecenyl acetate, pheromone analogs, fluorinated analogs.

INTRODUCTION

Sex pheromones mediate behavioral aspects of moth reproduction. Knowledge of how male moths detect pheromone is a topic of considerable interest because

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it might lead to development of ways to interfere with normal sex pheromone communication and suppress reproductive potential of pests. Recent hypotheses (Prestwich, 1993) concerning mechanisms involved in moth olfactory sensing of pheromones propose that the lipophilic pheromone enters olfactory sensilla on the male's antenna through pores in the cuticular wall of the sensilla and that the pheromone is solubilized in the hydrophilic lumen of the sensilla by binding with pheromone-binding protein (Vogt et al., 1991). The bound pheromone is transported across the sensillar lumen and transferred to a membrane-bound pheromone receptor protein (Vogt et al., 1988) on sensory dendrites within the sensillum. The binding of the pheromone with the receptor causes generation of an electrophysiological response in the dendrites that make direct input into the olfactory glomeruli of the moth central nervous system (Hansson et al., 1992) and result in the display of complex behavioral reactions by the male. The receptor-bound pheromone is then removed from the receptor and catabolized to ready the system for a fresh in-coming stimulus (Klun et al., 1992; Klun and Schwarz, 1993).

Organofluorine compounds have proven useful as probes in studies of the reactivity and modes of action of biologically important agonists such as pheromones and hormones (Welch, 1991). Due to its electronegativity, fluorine has a strong inductive electron-withdrawing effect that makes the chemical reactivity of bonds involving fluorine different from those involving hydrogen. Depending upon the extent and position of substitutions of hydrogen with fluorine in a molecule, fluorinated analogs can have dipole moments, solubilities, volatilities, and stability characteristics that are vastly different from compounds containing the usual carbon–hydrogen bonds. However, the fluorine atom is only 10% larger than the hydrogen atom, with a Van der Waals radius of 1.65 Å vs. 1.50 Å for hydrogen (Wenqi et al., 1993). Thus, fluorinated analogs are expected to have electronic characteristics that are different from the natural molecule but that possess space-filling qualities closely resembling the natural product. Substitution of fluorine for hydrogen in a biologically active substance and assay of the influence of such substitutions on activity can provide insight into the relative importance of electronic qualities of sites in the bioactive molecule as it interacts with charged sites within its complementary receptor.

Our long-standing interest in structure–activity relationships (Klun et al., 1992; Schwarz et al., 1990) in the female sex pheromone, 11-tetradecenyl acetate (11–14:OAc) (Figure 1, **1**), of the European corn borer (ECB), *Ostrinia nubilalis*, prompted us to investigate selectively fluorinated analogs of the pheromone. The purpose of this study was to learn how ECB males would respond when fluorine was substituted for hydrogen at specific sites in the pheromone molecule and to compare these responses with assay results obtained by other researchers who had evaluated the biological activity of similarly fluorinated sex pheromone analogs in other species of moths.

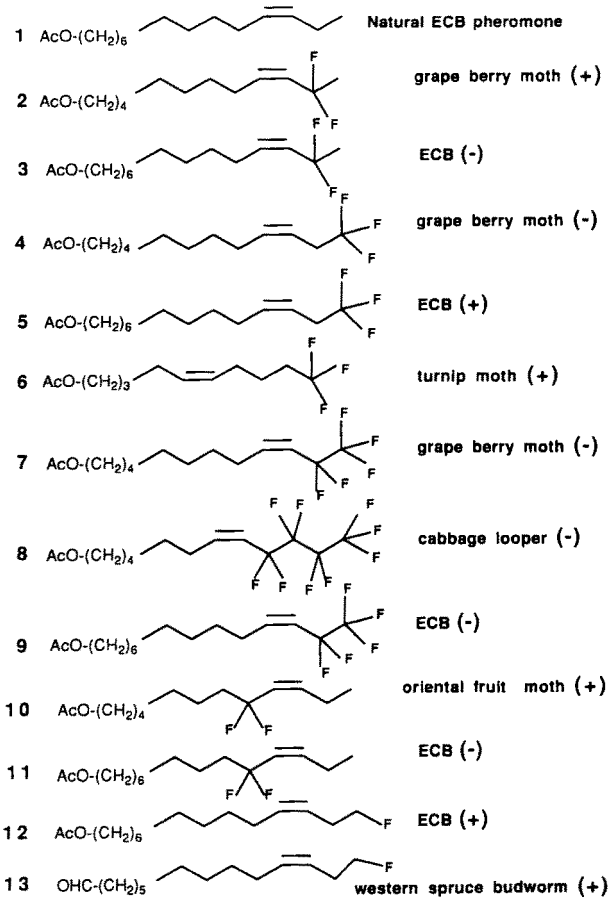

FIG. 1. The structure of partially fluorinated pheromone analogs of five species of moths and the European corn borer (ECB). The biological activities of analogs in the five species were determined and published previously by other researchers referred to in the text. (+) = analog mimicked natural pheromone. (−) = analog was not a pheromonal mimic. Structure **1** represents the natural ECB pheromone.

In the last few years many studies involving selectively fluorinated analogs of moth sex pheromones have been reported; however, previously no attempt had been made to develop a comparative overview of the effect of specific fluorine substitution patterns upon moth response across several species of moths. We surmised that such an overview might provide insight into whether the olfactory system of various species of moths that use chemically homologous

pheromones were structurally similar or dissimilar. We report results that show that these systems vary considerably among the moths.

METHODS AND MATERIALS

Rationale for Selecting Specific Fluorinated Pheromone Analogs

Five fluorinated ECB pheromone analogs (Figure 1; compounds **3, 5, 9, 11,** and **12**) were selected for study. The fluorine substitutions in the analogs were designed to be identical to substitution patterns in compounds that had been previously tested by others as pheromone mimics in species of moths that use acetate esters of monounsaturated long-chain fatty alcohols as pheromones. In general, fluorine was substituted for hydrogen atoms at either side of the double bond and on the terminal carbon atoms. Pheromone analog **2,** which had been studied (Bengtsson et al., 1990) in the grape berry moth (*Eupoecilia ambiguella*), had substitutions identical to our analog **3.** Our trifluoroanalog, **5,** was similar to pheromone analogs **4** and **6** tested against the grape berry moth (Bengtsson et al., 1990) and turnip moth (*Agrotis segetum*) (Wenqi et al., 1993), respectively. Pentafluoro-analog **9** was fluorinated like pheromone analogs **7** and **8,** which were the subjects of study with the grape berry moth (Bengtsson et al., 1990) and the cabbage looper moth (*Trichoplusia ni*) (Linn et al., 1992). ECB analog **11,** having two fluorine atoms internalized in the carbon chain next to the olefinic site was similar to **10,** which had been studied (Masnyk et al., 1989) in the Oriental fruit moth (*Grapholitha molesta*). The ECB analog, having a single fluorine atom on the terminal carbon, **12,** had a counterpart, **13,** that was studied in the western spruce budworm (*Choristoneura occidentalis*) (McLean et al., 1989). Analogs **1–7, 9, 11,** and **12** were evaluated for biological activity in field trapping tests. Some of them were also assayed using electrophysiological and/or flight tunnel techniques that confirmed their biological activities. Compounds **8, 10,** and **13** were assayed using flight tunnel or electrophysiological methods alone. In Figure 1, a positive sign follows the name of the insect if the corresponding fluorinated pheromone analog mimicked the species' natural pheromone. A negative sign is an indication that an analog lacked pheromonal activity.

Synthesis of Analogs

General. Gas chromatography on two Hewlett-Packard 5880A chromatographs fitted, respectively, with a polar 60-m × 0.25-mm DB-WAX (J & W Scientific, Folsom, California) and a nonpolar 50-m × 0.32-mm Ultra-1 columns (Hewlett-Packard, Palo Alto, California 94304) were used to determine the retention indices (Kováts et al., 1965) and chemical–geometrical purity of

the compounds. Infrared (IR) spectra were obtained on a Perkin-Elmer model 1320 spectrophotometer. Nuclear magnetic resonance (NMR) spectra were obtained using a GE QE300 with $CDCl_3$ as solvent and trimethylsilane for internal standard. The results are reported in δ values. Electron impact–mass spectra (EI-MS) were obtained using a Finnigan Mat INCOS 50 mass spectrometer.

Figure 2A shows the synthetic route we used to prepare (*Z*)-10,10-difluoro-11-tetradecenyl acetate **(11).** Details of the synthesis and spectral data for intermediates and the end product are as follows.

10-Undecenyl Tetrahydropyranyl (THP) Ether. To a stirred solution of 10-undecenyl-1-ol (40.0 g, 0.235 mol) and K-10 Montmorillonite clay (4.0 g) in diethyl ether (40 ml) was added dihydropyran (DHP) (23.6 g, 0.281 mol) during 45 min while the reaction temperature was kept below 30°C. After 2 hr, the reaction mixture was centrifuged. Most of the solvent was removed on a rotary evaporator and the residue was distilled at 106–110°C (0.14 mm); the yield was 50.16 g (84% of theory). IR 3069 (m), 2929 (s), 2853 (s), 1639 (m), 1464 (m), 1454 (m), 1443 (m), 1347 (m), 1320 (w), 1258 (w), 1199 (m), 1118 (s), 1073, 1027 (s), 988 (m), 902 (m), 866 (s), 809 (w).

10-(Tetrahydropyranyloxy)decanal. To a mechanically stirred mixture of 10-undecenyl THP ether (30 g, 0.12 mol), osmium tetroxide (250 mg, 1.2 mmol in 10 ml *t*-butyl alcohol), dioxane (360 ml), and water (120 ml) was added sodium periodate (60 g, 0.28 mol) during 45 min, while keeping the reaction

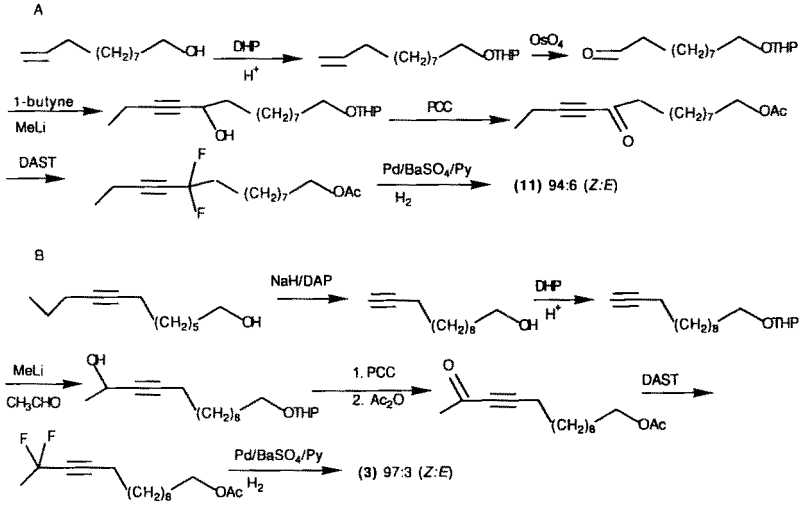

FIG. 2. Schemes for synthesis of pheromone analogs **11** and **3**: (A) preparation of **11** and (B) preparation of **3**.

temperature below 30°C. The mixture was stirred for another 1.5 hr, water (200 ml) and petroleum ether (50 ml) were added, swirled vigorously, and the ether phase was removed. Extraction was repeated three more times; the combined extract was extracted once with aqueous Na_2SO_3, twice with water, and dried over anhydrous $MgSO_4$. The product was purified by flash chromatography using petroleum ether–methyl tertiary butyl ether (MTBE)–$CHCl_3$ (80:10:10). The yield was 16.6 g (57% of theory). IR 3004 (s), 2921 (s), 2851 (s), 2706 (w), 1722 (s), 1464 (m), 1453 (m), 1443 (m), 1350 (m), 1199 (m), 1118 (s), 1072 (s), 1029 (s), 968 (m), 899 (m), 865 (w), 750 (s). ^{1}H NMR 9.765 CHO (t, J = 1.5, H1), 2.423 (t, J = 7.2; d, J = 1.5, H2), 1.305 [$-(CH_2)_x-$].

14-Tetrahydropyranyloxy-3-tetradecyn-5-ol. To a stirred solution of 1-butyne (3.2 g, 50 mmol) in 70 ml tetrahydrofuran (THF), cooled in an ice–salt bath, was added methyllithium (25 ml of 1.4 M in ether, 35 mmol) at such a rate that the reaction temperature was kept below 0°C. The mixture was stirred for 30 min, then cooled to −78°C and a solution of 10-(tetrahydropyranyloxy)decanal (7.48 g, 29 mmol) in THF (20 ml) was added while the reaction temperature was kept below −70°C. After 1.5 hr, the reaction mixture was poured into water, acidified, and worked up to yield a pale green oil that was purified by flash chromatography using petroleum ether–MTBE–$CHCl_3$ (75:15:10) to obtain a clear, colorless oil in a yield of 3.40 g (38% of theory). IR 3394 (s), 2915 (s), 2860 (s), 1460 (m), 1355 (m), 1320 (m), 1260 (m), 1120 (s), 1070 (s), 1020 (s), 900 (m), 865 (m), 785 (s), 630 (m). ^{1}H NMR 1.238 (t, J = 7.4, H1), 2.226 (t, J = 7.4, d, J = 1.5, H2), 4.340 (t, J = 6.6, further small splitting, H5), 1.302 [$-(CH_2)_x-$].

14-Tetrahydropyranyloxy-3-tetradecyn-5-one. A mixture of 14-tetrahydropyranyloxy-3-tetradecyn-5-ol (4.0 g, 12.9 mmol) and pyridinium chlorochromate (PCC) (11 g, 51 mmol) in methylene chloride (60 ml) was allowed to stand for 1 hr, and then it was diluted with diethyl ether (80 ml). The solution was decanted from the gummy black precipitate and passed through a column of Florisil (60 g), followed by a mixture of methylene chloride–ether (40:60) (200 ml). Solvent was removed and product was purified by flash chromatography using hexane–MTBE–(90:10). The yield of clear, colorless oil was 2.03 g (50% of theory). IR 2920 (s), 2852 (s), 2205 (s), 1665 (s), 1453 (m), 1349 (m), 1313 (m), 1254 (m), 1233 (m), 1198 (m), 1160 (m), 1134 (s), 1117 (s), 1072 (s), 1024 (s), 986 (m), 892 (w), 865 (w), 808 (w).

14-Acetoxy-3-tetradecyn-5-one. A mixture of 14-tetrahydropyranyloxy-3-tetradecyn-5-one (1.73 g, 5.6 mmol), acetyl chloride (0.5 ml), acetic anhydride (Ac_2O) (5 ml), and acetic acid (5 ml) was allowed to stand for two days at room temperature, then poured into stirred aqueous $NaHCO_3$ containing ice. After work-up the product was purified by flash chromatography using petroleum ether–MTBE (85:15). Impure fractions were combined and rechromatographed to yield a total of 0.60 g (40% of theory) of colorless oil. IR 2924 (s), 2853

(s), 2204 (s), 1732 (s), 1663 (s), 1458 (m), 1381 (m), 1361 (s), 1311 (m), 1235 (s), 1159 (s), 1030 (m). ^{1}H NMR 1.212(t, J = 7.5, H1), 2.378(q, J = 7.5, H2), 2.523(t, J = 7.5, H6), 1.298(br.s, H7–H13), 4.049(t, J = 6.6, H14), 2.049(s,CH_3COO-).

10,10-Difluoro-11-tetradecynyl Acetate. To a stirred solution of diethylaminosulfur trifluoride (DAST) (3.6 g, 22 mmol) in 1,2-dichloroethane (20 ml) at room temperature was added 14-acetoxy-3-tetradecyn-5-one (600 mg, 2.3 mmol). The reaction flask was placed in a bath at 60°C. At 6 hr and at 9 hr, 1.2 g (1 ml) portions of DAST were added. After 30 hr, the cooled reaction mixture was poured onto an aqueous solution of $NaHCO_3$ containing ice and worked up. Flash chromatography with petroleum ether–$CHCl_3$ (70:30) yielded 120 mg (18.5%) of yellow oil. IR 2925 (s), 2853 (s), 2249 (m), 1729 (s), 1460 (m), 1361 (m), 1317 (m), 1231 (s), 1163 (m), 1029 (m). ^{1}H NMR 1.183 (t, J = 7.5, H14), 2.287 (m, H2), 1.90–2.02 (m, H9), 1.311 (s, H2–H8), 4.050 (t, J = 6.76, H1), and 2.050 (s,CH_3COO-).

(Z)-10,10-Difluoro-11-tetradecenyl Acetate ***(11).*** 10,10-Difluoro-11-tetradecynyl acetate (100 mg, 0.35 mmol) in pyridine (2 ml) was hydrogenated at atmospheric pressure in the presence of 5% Pd on $BaSO_4$ (300 mg) until the theoretical amount of hydrogen was taken up. The reaction mixture diluted with petroleum ether was filtered through a column of Florisil (5 g) and the pyridine removed by aqueous washing. Flash chromatography on silica gel with petroleum ether–MTBE (85:15) followed by short-path distillation at 0.05 mm pressure and bath temperature of 130°C yielded 80 mg (80%) of clear colorless liquid. Gas chromatography showed that it was a 97:3 mixture of (*Z*)- and (*E*)-10,10-difluoro-11-tetradecenyl acetate. IR 2926 (s), 2855 (s), 1734 (s), 1658 (w), 1463 (m), 1408 (w), 1362 (m), 1233 (s), 1163 (w), 1029 (m). ^{1}H NMR 1.009 (t, J = 7.5, H14), 2.246 (d, J = 9.1, q, J = 7.5, H13), 5.646 (t, J = 9.1, d, J = 10.8, H12), 5.417 (t, J = 16.2, d, J = 10.8, H11), 1.889 (m, H9), 1.303 (br.s, H2–H8), 4.051 (t, J = 6.6, H1), 2.044 (s, CH_3COO-). EI-MS 250 (0.5, M^+-2HF), 210 (0.4 M^+-60), 181 (0.6), 167 (1), 161 (2), 153 (3), 147 (2), 139 (3), 133 (4), 132 (2), 128 (6), 122 (4), 121 (5), 119 (3), 113 (9), 112 (16), 111 (7), 108 (7), 105 (10), 100 (12), 98 (14), 97 (17), 95 (15), 94 (41), 85 (16), 82 (12), 81 (20), 77 (22), 69 (23), 67 (25), 61 (14), 55 (54), 43 (100).

Synthesis of (*Z*)-13,13-Difluoro-11-tetradecenyl acetate **(3)** is shown schematically in Figure 2B. Details for synthesis of the compound are as follows.

11-Dodecyn-1-ol. To NaH (12.5 g, 0.52 mol) was added 1,3-diaminopropane (150 ml, freshly distilled from CaH_2) and the bath temperature was raised to 75°C. When hydrogen evolution subsided after 2 hr, 7-dodecyn-1-ol (29 g, 0.16 mol) was added and after 40 min the mixture was heated to 100°C for 2 hr. The reaction was worked up by partitioning between petroleum ether and

water and used for the preparation of 11-dodecynyl THP ether without further purification.

11-Dodecynyl THP Ether. To the stirred red solution of crude 11-dodecyn-1-ol, obtained above, was added K-10 Montmorillonite clay (64 g) and DHP (17 g, 0.20 mol). After 10 hr, the clay was removed by centrifugation and rinsed twice with petroleum ether. The combined petroleum ether solution was concentrated and distilled. 11-Dodecynyl THP ether had a bp 119–125°C (0.60–0.10 mm). The yield was 25.65 g (58% of theory). IR 3309 (m), 2916 (s), 2853 (s), 2112 (w), 1451 (m), 1347 (m), 1320 (w), 1256 (w), 1116 (s), 1071 (s), 1020 (s), 978 (m), 898 (w), 865 (w), 809 (w), 616 (m). ^{1}H NMR 1.949 (t, J = 2.9, H12), 2.180 (t, J = 7.2, d, J = 2.9, H9), 1.207 (br.s, H2–H8).

14-Tetrahydropyranyloxy-3-tetradecyn-2-ol. To a solution of 11-dodecynyl THP ether (15.0 g, 56 mmol) in THF (100 ml), cooled in an ice–salt bath, was added methyllithium (52 ml of 1.4 M in ether, 73 mmol) during 10 min while keeping the reaction temperature below 0°C. The mixture was allowed to stand at −10°C for 30 min, cooled to −78°C, and acetaldehyde (3.7 g, 84 mmol) was added during 15 min while the temperature was kept below −60°C. When addition was completed, the cooling bath was removed and the reaction mixture was allowed to stand at room temperature for 20 hr. The reaction was worked up in the usual manner and the product was purified by flash chromatography using petroleum ether–MTBE–$CHCl_3$ (70:20:10). The yield of product was 12.37 g (71% of theory). IR 3418 (m), 2921 (s), 2853 (s), 2239 (w), 1443 (m), 1348 (m), 1320 (m), 1275 (w), 1258 (w), 1131 (m), 1116 (m), 1071 (m), 1020 (m). ^{1}H NMR 1.429 (d, J = 6.6, H1), 4.514 (q, J = 6.6, t, J = 1.9, H2), 2.188 (t, J = 7.0, d, J = 1.9, H5), 1.234 (br.s, H6–H15).

14-Tetrahydropyranyloxy-3-tetradecyn-2-one. A mixture of 14-tetrahydropyranyloxy-3-tetradecyn-2-ol (8.0 g, 25.8 mmol) and PCC (22 g, 102 mmol) in methylene chloride (120 ml) was stirred for 2 hr while the reaction temperature was kept below 30°C. The reaction mixture was diluted with diethyl ether and the gummy precipitate washed with additional ether. The combined organic layer was passed through Florisil (60 g) followed by a mixture of methylene chloride–MTBE (40:60) (400 ml). After the solvent was removed, the residue was purified by flash chromatography using petroleum ether–MTBE (85:15). The yield of clear colorless oil was 5.45 g (68% of theory). IR 3332 (w), 2920 (s), 2850 (s), 2200 (s), 1666 (s), 1453 (m), 1352 (s), 1320 (s), 1220 (s), 1200 (s), 1118 (s), 1072 (s), 1020 (s), 964 (m), 899 (m), 864 (m), 807 (m), 713 (w), 650 (w). ^{1}H NMR 2.323 (s, H1), 2.350 (t, J = 6.9, H5). 1.287 (br.s, H6–H13).

14-Acetoxy-3-tetradecyn-2-one. 14-Tetrahydropyranyloxy-3-tetradecyn-2-one (5.0 g, 16.2 mmol), acetyl chloride (0.5 ml), acetic anhydride (15 ml), and acetic acid (15 ml) were mixed and heated in an oil bath at 60°C for 1.5 hr. The deep red reaction mixture was poured into a stirred mixture of aqueous $NaHCO_3$ and ice and worked up with petroleum ether. The product was purified

by flash chromatography using petroleum ether–MTBE (90:10) to yield 2.74 g (64% of theory) of a clear, colorless oil. IR 3331 (w), 2918 (s), 2857 (s), 2202 (s), 1726 (s), 1669 (s), 1458 (m), 1426 (m), 1359 (s), 1226 (s), 1034 (s), 960 (m), 926 (m). ^{1}H NMR 2.323 (s, H1), 2.353 (t, J = 6.9, H5), 1.290 (br.s, H6–H12), 1.587 (m, H13), 4.052 (t, J = 6.8, H14), 2.049 (s, CH_3COO-).

13,13-Difluoro-11-tetradecynyl Acetate. To a stirred solution of 14-acetoxy-3-tetradecyn-2-one (2.3 g, 9.6 mmol) in methylene chloride (50 ml) was added DAST (13.9 g, 86 mmol, 11.4 ml) and kept at 60°C for 25 hr. The reaction mixture was poured into aqueous $NaHCO_3$ containing ice, worked up, and purified by flash chromatography using petroleum ether–MTBE (92:8) to yield a pale yellow oil (0.82 g, 33% of theory). IR 2917 (s), 2853 (s), 2243 (s), 1726 (s), 1639 (w), 1443 (m), 1380 (s), 1363 (s), 1228 (s), 1159 (s), 1126 (s), 1030 (m), 908 (m). ^{1}H NMR 1.842 (t, J = 20.1, H14), 2.51 (m, H10), 1.288 (br.s, H2–H9), 4.056 (t, J = 6.8, H1), 2.048 (s, CH_3COO-).

(Z)-13,13-Difluoro-11-tetradecenyl Acetate ***(3).*** 13,13-Difluoro-11-tetradecynyl acetate (300 mg, 1.0 mmol) and 5% Pd on $BaSO_4$ (600 mg) were placed in pyridine (Py) (5 ml) and hydrogenated at atmospheric pressure. The reaction mixture was centrifuged to remove catalyst and worked up to yield 280 mg (93% of theory) of clear, colorless oil. GC analysis indicated that it was a 94:6 mixture of (*Z*)- and (*E*)-13,13-difluoro-11-tetradecenyl acetate. IR 2924 (s), 2853 (s), 1733 (s), 1656 (m), 1447 (m), 1381 (m), 1362 (m), 1228 (s), 1152 (s), 1123 (s), 1031 (s), 904 (s). ^{1}H NMR 1.702 (t, J = 18.0, H14), 5.504 (q, J = 12.0, H12), 5.678 (m, H11), 2.241(m, H10), 1.276 (br.s, H2–H9), 4.050 (t, J = 6.8, H1), 2.047 (s, CH_3COO-). EI-MS 251 (0.7), 250 (2, M^+-2HF), 207 (0.6), 175 (0.6), 161 (2), 153 (1), 150 (1), 139 (2), 135 (3), 133 (3), 125 (3), 124 (4), 123 (2), 121 (4), 112 (8), 109 (7), 108 (6), 99 (8), 96 (9), 95 (18), 86 (30), 83 (14), 82 (23), 81 (25), 73 (20), 69 (27), 67 (30), 55 (45), 43 (100).

14,14,14-Trifluoro-11-tetradecenyl Acetate ***(5).*** The synthesis of **5** is shown schematically in Figure 3A. 3,3,3-Trifluoropropyltriphenylphosphonium bromide (Ullmann and Hanack, 1989) (1.1 g, 2.5 mmol in 20 ml THF; 2.7 mmol)

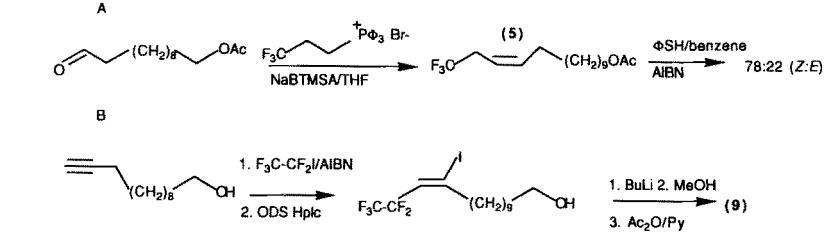

FIG. 3. Schemes for synthesis of **5** and **9:** (A) preparation **5** and (B) preparation of **9.**

was added to 2.7 ml of a 1 M THF solution of sodium bistrimethylsilylamide (2.7 mmol). After 0.5 hr, the solution was cooled to −78°C and 11-oxoundecyl acetate (Schwarz et al., 1986) (0.625 g, 2.7 mmol) was added. The reaction mixture was warmed to 0°C and held at that temperature overnight. Work-up in the usual manner and flash chromatography (ethyl acetate–hexane, 7.5:92.5) yielded 0.4 g (50% of theory) product. GC analysis on the polar and nonpolar capillaries showed a single compound that was presumed to be the *Z* isomer. Isomerization of 0.1 g of the product using 2,2′-azobis(2-methylpropionitrile) (AIBN)–thiophenol in benzene (Schwarz et al., 1986) gave rise to a 80:20 *E*:*Z* mixture. The two peaks had identical mass spectra. IR for *Z* 3015 (m), 2915 (s), 2880 (s), 1740 (s), 1650 (w), 1480 (m), 1370 (m), 1350 (s), 1250 (s), 1130 (s). ^{1}H NMR 1.28 [br. s, $-(CH_2)_x-$], 1.603 (m, H2), 2.044 (br. s, H10 and CH_3COO-), 2.839 (m, H13), 4.051 (t, J = 6.6, H1), 5.377 (t, J = 10.8, t, J = 6.9, d, J = 1.5, H12), 5.723 (m, H11). EI-MS 284 (1), 214 (4), 213 (2), 200 (4), 199 (3), 173 (1), 145 (1), 138 (10), 127 (3), 124 (2), 123 (2), 116 (4), 111 (1), 110 (3), 109 (9), 103 (3), 98 (3), 97 (8), 96 (11), 95 (15), 85 (2), 84 (6), 83 (21), 82 (19), 81 (18), 79 (2), 77 (4), 73 (6), 71 (5), 70 (11), 69 (37), 68 (14), 67 (21), 65 (2), 61 (37), 59 (2), 58 (3), 57 (13), 56 (17), 55 (52), 54 (13), 53 (3), 44 (3), 43 (100), 42 (14), 41 (37).

14,14,14,13,13-Pentafluoro-11-tetradecenyl Acetate ***(9)***. Figure 3B shows the preparation of **9**. The synthesis was carried out by a reaction sequence that was identical to the one reported by Sun and Prestwich (1990). The (*Z*)-iodide resulting from the addition of pentafluoroethyl chloride to 11-dodecyn-1-ol was separated from the minor *trans*-isomer by reverse phase HPLC. The desired (*Z*)-14,14,14,13,13-pentafluoro-11-tetradecen-1-ol was obtained after metal–halogen exchange with *n*-butyl lithium and subsequent quenching with methanol. The crude reaction product was acetylated using acetyl chloride–pyridine and purified by flash chromatography with a 96.5:3.5 hexane–ethyl acetate mixture. A portion of the compound was isomerized using thiophenol–AIBN in benzene (Schwarz et al., 1986) to yield an 80:20 *E*:*Z* mixture which was used to make the appropriate 97:3 *Z*:*E* mixture for bioassay purposes. IR 3315 (w), 2915 (s), 2845 (s), 1740 (s), 1660 (s), 1555 (w), 1505 (w), 1465 (s), 1455 (s), 1410 (w), 1385 (m), 1365 (s), 1335 (s), 1220 (s), 1100 (s), 1050 (s), 740 (s), 635 (m), 605 (m). ^{1}H NMR 1.28 [br. s, $-(CH_2)_x-$], 1.419 (m, H9), 1.619 (m, H2), 2.045 (s, CH_3COO-), 2.321 (br. s, H10), 4.052 (t, J= 6.6, H1) 5.460 (m, H12), 6.107 (t, J = 9.9, t, J = 7.8, d, J = 2.1, H11). EI-MS 284 (1), 214 (4), 213 (2), 200 (4), 199 (3), 173 (1), 145 (1), 138 (10), 127 (3), 124 (2), 123 (2), 116 (4), 111 (1), 110 (3), 109 (9), 103 (3), 98 (3), 97 (8), 96 (11), 95 (15), 85 (2), 84 (6), 83 (21), 82 (19), 81 (18), 79 (2), 77 (4), 73 (6), 71 (5), 70 (11), 69 (37), 68 (14), 67 (21), 65 (2), 61 (37), 59 (2), 58 (3), 57 (13), 56 (17), 55 (52), 54 (13), 53(3), 44 (3), 43 (100), 42 (14), 41 (37).

14-Fluoro-11-tetradecenyl Acetate. The synthesis of the geometrical iso-

mers of this compound was reported previously (Schwarz et al., 1990). The isomers were mixed to make a 97:3 *Z*:*E* mixture for the field testing. ^{1}H NMR 1.276 [br. s, $-(CH_2)_x-$], 1.615 (m, H2), 2.045 (br. s, H10 and CH_3COO-), 2.462 (m, H13), 4.052 (t, J = 6.9, H1), 4.418 (t, J = 6.6, d, J = 47.1, H14), 5.368 (m, H12), 5.538 (m, H11). EI-MS: 212 (M^+-60; 6), 192 (3), 164 (1), 163 (1), 150 (1), 149 (3), 141 (2), 138 (2), 137 (1), 136 (3), 135 (7), 128 (3), 127 (3), 124 (5), 123 (4), 122 (4), 121 (10), 115 (1), 114 (9), 113 (3), 111 (2), 110 (13), 109 (10), 108 (5), 107 (5), 101 (3), 100 (34), 99 (3), 98 (1), 97 (6), 96 (24), 95 (22), 94 (10), 93 (8), 91 (2), 87 (2), 86 (4), 85 (15), 84 (2), 83 (3), 82 (41), 80 (16), 79 (11), 77 (2), 73 (6), 72 (2), 71 (2), 70 (3), 69 (24), 68 (25), 67 (44), 66 (5), 65 (2), 61 (11), 59 (7), 57 (7), 56 (8), 55 (52), 54 (25), 53 (8), 47 (2), 44 (4), 43 (100), 42 (14), 41 (68).

Field Tests

We used insect traps to bioassay the analogs. Tests were conducted in corn fields near Beltsville, Maryland, during summer flights of the ECB. Three pheromonal forms of ECB (Klun and Huettel, 1988) exist in the Beltsville area, and they communicate by using geometric blends of 11-14:OAc; ca. 97:3, 40:60, or 3:97 *Z*:*E*. The form using 97:3 *Z*:*E* is most abundant in the area. Therefore, the isomeric composition of the analogs was adjusted to target that form of the moth. Analogs **3** and **11** had 94:6 and 97:3 *Z*:*E* proportions, respectively, as result of the Lindlar reduction, and they were used as such. Exact 97:3 *Z*:*E* geometric mixtures of the other analogs were prepared for the field tests by mixing purified isomers or by mixing *Z*:*E* with pure *Z* isomer. All compounds used in the field tests were purified (95–98%) by flash chromatography (Still et al., 1978), reverse-phase chromatography using a 31-cm × 10-mm-ID column packed with 5-μm ODS Spherisorb (Rainin Instrument Co., Inc., Emeryville, California 94608) eluted with acetonitrile–water (9:1) at 2.5 ml/min or by argentation chromatography using two 31-cm × 10-mm-ID columns packed with 20% $AgNO_3$-impregnated (w/w) 5-μm Spherisorb silica plumbed in series and eluted with toluene pumped at 5 ml/min. The first field test compared responses of males to the natural pheromone **(1)** versus a monofluorinated analog **(12).** The second test compared male responses to the pheromone and four analogs **(3, 5, 9,** and **11).** For all tests, heptane solutions of the compounds (ca. 10 μg/μl) were prepared and 100 μg of each compound was applied individually to red rubber serum bottle stoppers (#1780J07, Thomas Scientific, Swedesboro, New Jersey 08085), which were placed randomly in cone-shaped traps (Webster et al., 1986) positioned at the perimeter of corn fields with 30 m between traps. Nightly ECB male captures in traps baited with analogs were compared to captures in traps baited with the natural pheromone. New lures were placed in the traps nightly, and all tests were conducted using a randomized complete

block design with replication within and over nights. Trap capture data were analyzed as a three factor model: treatment, night, and replicate. Treatment was a fixed effect, while night and replicate were random effects. To meet the assumptions of the general linear model, the $\ln(y + 1)$ transformation was used on the dependent variable (number of moths captured). Statistical analysis was done on the transformed data using the SAS PROC GLM (SAS Institute, 1989).

RESULTS AND DISCUSSION

Gas chromatography of the ECB pheromone and its analogs showed (Table 1) that only the polyfluorinated analog **9** was significantly more volatile than the pheromone **1.** This was consistent with the observation of Prestwich et al. (1990) that perfluoroalkyl compounds were always more volatile than their corresponding hydrocarbon analogs. The volatilities of the difluorosubstituted **11** and **3** and the trifluorosubstituted **5** were only slightly different from the pheromone **1.** The monofluorinated compound **12** was least volatile of all compounds in the set based upon the 2408 retention index on the Ultra 1 column. At the same time, the compound displayed significantly greater polarity than all other compounds based upon its 1871 retention index on DB-WAX. The chromatographic data reflect the physical–chemical effects caused by substitution of hydrogen with fluorine in a molecule.

Bioassay results reported in Table 2 show that traps baited with 14,14,14-trifluoro-11-14:OAc **(5)** and 14-monofluoro-11-14:OAc **(12)** proved to be equivalent to the natural pheromone in causing capture of ECB males. This was true although the volatility and polarity of **5** and **12** were different from each another and the pheromone **1.** On the other hand, 13,13-difluoro-11-14:OAc **(3),** 10,10-difluoro-11-14:OAc **(11),** and 13,13,14,14,14-pentafluoro-11-

TABLE 1. RETENTION INDICES FOR EUROPEAN CORN BORER SEX PHEROMONE AND FLUORINATED PHEROMONE ANALOGS ON POLAR (DB-WAX) AND NONPOLAR (ULTRA 1) CHROMATOGRAPHIC COLUMNS

Compound	Retention index	
	DB-WAX	Ultra 1
13,13,14,14,14-Pentafluoro-Z-11-14:OAc **(9)**	1656	1964
14,14,14-Trifluoro-Z-11-14:OAc **(5)**	1733	2141
Z-11-14:OAc **(1)**	1786	2154
10,10-Difluoro-Z-11-14:OAc **(11)**	1802	2296
13,13-Difluoro-Z-11-14:OAc **(3)**	1794	2303
14-Monofluoro-Z-11-14:OAc **(12)**	1871	2408

TABLE 2. MEAN NIGHTLY MALE ECB TRAP-CAPTURE RATES OF TRAPS BAITED WITH SYNTHETIC ECB FEMALE SEX PHEROMONE AND FLUORINATED ANALOGS[a]

Test	Compound	Mean males/trap/nigl
1 (N = 24)	11-14:OAc **(1)**	7.5a
	14-monofluoro-11-14:OAc **(12)**	7.3a
2 (N = 25)	11-14:OAc **(1)**	19.4a
	14,14,14-trifluoro-11-14:OAc **(5)**	16.7a
	10,10-difluoro-11-14:OAc **(11)**	1.1b
	13,13-difluoro-11-14:OAc **(3)**	1.5b
	13,13,14,14,14-pentafluoro-11-14:OAc **(9)**	0.7b

[a]Means reported within tests followed by the same letter are not significantly different from one anoth according to Sidak t tests (Sidak, 1967). Alpha = 0.05. N = number of replicates.

14:OAc **(9)** were essentially inactive as pheromone mimics. The potency of **5** and **12** as pheromone mimics shows that these analogs fulfilled a requirement for a 14-carbon chain in the pheromone molecule and that the fluorination of the terminal carbon did not interfere with a productive interaction with the ECB chemoreceptive system. Comparison of the biological activity of **5** with identically substituted analogs of the pheromones of the grape berry moth **(4)** and the turnip moth **(6)** (Figure 1) shows that trifluoro substitution in the terminal carbon of the pheromone destroyed activity in case of the grape berry moth but not in case of the turnip moth. Retention of pheromonal activity in the monofluorinated ECB analog **12** was like the result obtained with the western spruce budworm pheromone analog **(13),** where pheromonal activity was retained with monofluorination of the terminal carbon. Substitution of hydrogen on either side of the double bond with fluorine (compounds **3** and **11**) resulted in loss of ECB biological activity. However, substitution of the hydrogen on either side of the double bond in pheromones of the grape berry moth **(2)** and oriental fruit moth **(10)** was permissible. It did not adversely affect pheromonal activity in those species. Multiple fluorination of carbon atoms beyond the double bond in the ECB pheromone **(9)** caused a loss of pheromonal activity for the analog. This effect was the same as was observed when all hydrogen beyond the double bond in pheromones of the grape berry **(7)** and the cabbage looper **(8)** moths were substituted with fluorine. Previous studies with fluorinated methanes (Welch, 1991) have shown that C—F bond length shortens and bond strength increases with increased fluorination, and it is reasonable that these effects and increased electron density associated with polyfluorination contribute to a loss of pheromone activity in grape berry, cabbage looper, and ECB moths.

Clearly, introduction of the same or similar fluorination patterns into a pheromone molecule have unpredictable effects on moth responses from one

species to another. This is evidence that the importance of the electronic qualities at specific sites within a pheromone molecule differs from one species to another. Correspondingly, the complementary sites for interaction of pheromone with binding proteins, receptor proteins, and/or catabolic enzymes that comprise the olfactory chemosensory systems of the moths must also differ structurally from species to species. This idea is reinforced by the work of Chapman et al. (1978) and Bestmann et al. (1992). Chapman et al. found that despite the fact that the red-banded leaf roller (*Argyrotaenia velutinana*) and the ECB use an identical compound as sex pheromone, their chemoreceptors for it have different chirality. Bestmann et al. (1992) have developed evidence to show that the pheromonal receptor systems of *Bombyx mori* and *Manduca sexta* are structurally different, although they use the same compound as pheromone. Their research also showed that a similar situation exists in *Antherea pernyi* and *A. polyphemus*. Thus, there is compelling evidence to conclude that the components of the olfactory systems of moths are structurally dissimilar notwithstanding that they are responsible for detection of similar or identical pheromonal compounds.

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