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BIOISOSTERIC APPROACH TO ELUCIDATION OF BINDING OF THE ACETATE GROUP OF A MOTH SEX PHEROMONE COMPONENT TO ITS RECEPTOR

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Abstract—A number of analogs of (*Z*)-5-decenyl acetate, a pheromone component of the turnip moth, *Agrotis segetum*, in which the acetate group has been replaced by functional groups that may function as bioisosters, have been synthesized and tested using single-cell electrophysiology. The activities have been interpreted in terms of the molecular electrostatic potentials of the polar functional group as calculated by *ab initio* quantum mechanical calculations. It is concluded that both oxygens of the acetate group in (*Z*)-5-decenyl acetate contribute to the interactions between the pheromone component and its receptor. Furthermore, the results indicate that the crucial interaction between the carbonyl group and the receptor, which is most probably a hydrogen bonding interaction, takes place in a direction pointing away from the hydrocarbon chain of the pheromone component.

Key Words—Lepidoptera, Noctuidae, *Agrotis segetum*, (*Z*)-5-decenyl acetate, pheromone analogs, structure-activity, bioisosteres, single-sensillum recordings, receptor interaction, molecular electrostatic potential, quantum mechanical calculations, *ab initio* calculations.

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INTRODUCTION

A model for the interaction between (Z)-5-decenyl acetate (**1**), a pheromone component of the turnip moth, *Agrotis segetum*, and its receptor has previously been developed, and a number of structure–activity studies based on this model have been reported (Liljefors et al., 1985, 1987; Bengtsson, 1988; Bengtsson et al., 1987, 1990; Jönsson et al., 1991 a,b, 1992, 1993; Gustavsson et al., 1995). The proposed bioactive conformation of **1** is shown in Figure 1. In the development of the model, three pharmacophore elements, i.e., structural elements of decisive importance for the activity of **1**, have been identified: the terminal methyl group, the double bond, and the acetate group. Due to its polarity and hydrogen-bonding capability, the acetate group should be of great importance for the recognition and binding of the pheromone component to its receptor. Thus, it is of interest to investigate the details of the interaction between the acetate group and the receptor. In the present study, a number of analogs of (Z)-5-decenyl acetate have been synthesized and tested by single-cell electrophysiology. In these analogs, the acetate group has been replaced by a functional group that mimics some aspect of the electrostatic properties of the acetate group and that may function as a bioisostere to the acetate group. The purpose of this study was to find out how the acetate group and the receptor interact. In particular, we were interested in studying the directions in which the interactions between the acetate group and the receptor take place. It is reasonable to assume that the acetate group is interacting with the receptor *via* hydrogen bonding involving one or both oxygen atoms. Various possible sites and directions of hydrogen bond interactions are shown in Figure 2.

Previously, modifications of the acetate group of (Z)-7-dodecenyl acetate, another pheromone component of *Agrotis segetum*, were studied by single-cell

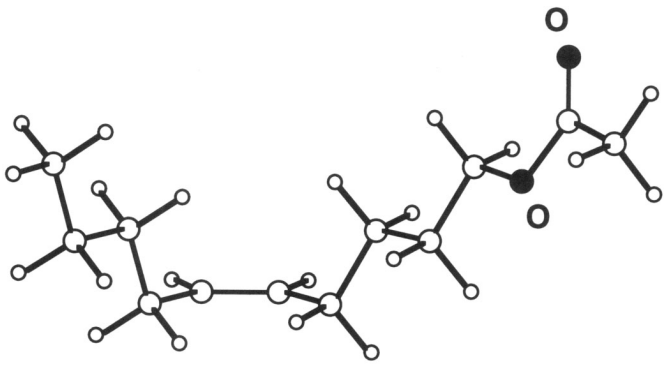

FIG. 1. (Z)-5-Decenyl acetate in its proposed bioactive conformation.

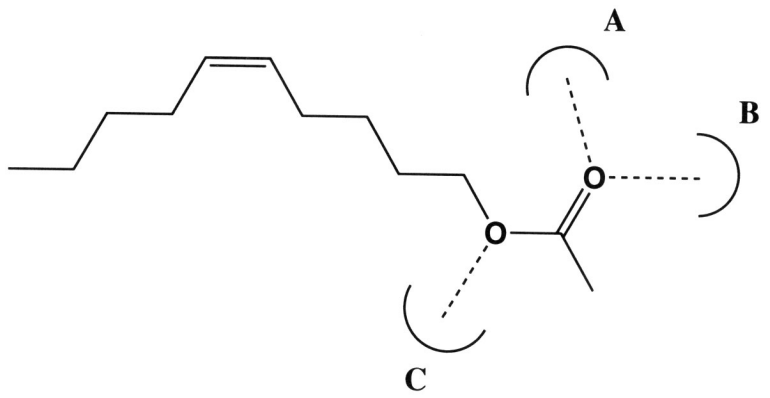

FIG. 2. Possible hydrogen bonding sites and directions for the acetate group of **1**.

electrophysiology (Liljefors et al., 1984). The results from that study imply that, although the presence of the carbonyl group is crucial for the activity, both oxygens in the ester function of (*Z*)-7-dodecenyl acetate are important for full biological activity. In a recent study by Hoskovec et al. (1996), mimics of the acetate function in a pheromone component of the Oriental fruit moth, *Cydia molesta*, (*Z*)-8-dodecenyl acetate, were investigated. The acetate group was replaced with a chloroformate and a lactone function. These authors found that the chloroformate and the alkene-4-olide functional group successfully mimicked the acetate function of the natural pheromone component.

Priesner et al. (1975) and Tamaki et al. (1984) also studied acetate group analogs of pheromone components of various moth species. However, the biological assays used in those studies, EAG and field trapping, preclude drawing conclusions about interactions with a specific receptor.

The analogs studied in the present work are shown in Figure 3. These analogs were selected on the basis of electrostatic potential maps calculated by quantum mechanical *ab initio* calculations. Warthen et al. (1995) recently employed calculated electrostatic potentials in a structure–activity study on (*Z*)-11-tetradecen-1-ol acetate and a series of fluoro analogs.

Bioisosteric replacement of an ester by a heterocyclic analog as in compounds **5** and **6** has to our knowledge not previously been attempted for pheromone components. However, such replacements have been successful in other areas. For instance, Orlek et al. (1991) studied the replacement of an ester group by heterocyclic analogs in compounds interacting with the muscarinic acetylcholine receptor and found that an oxadiazole could successfully replace an ester function *in vitro*. Furthermore, Brown et al. (1996) in a study of analogs of the antibacterial agent pseudomonic acid, replaced an ester function by an oxazole

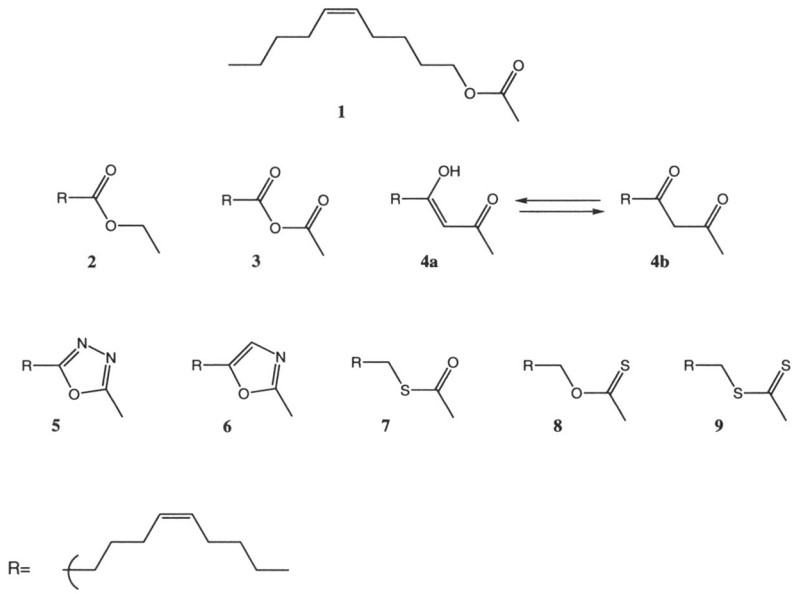

FIG. 3. Structure of compounds synthesized and studied. Names of compounds are in text.

in order to avoid ester hydrolyses *in vivo*. For a recent review on bioisosterism see Patani and LaVoie (1996).

METHODS AND MATERIALS

Synthesis. All reactions of air- and water-sensitive materials were performed under a nitrogen or argon atmosphere with oven-dried glassware. Anhydrous diethyl ether (ether) and tetrahydrofuran (THF) were distilled prior to use from dark blue solutions of the sodium benzophenone radical anion. *N*,*N*′-Dimethyl-*N*,*N*′-propylene urea (DMPU), purchased from Fluka Chemie AG, and toluene was distilled under vacuum from calcium hydride. The dimethyl sulfide complex of cuprous bromide was prepared according to House et al. (1975).

Thin-layer chromatography (TLC), on silica gel 60 F254, was used for the monitoring of the reactions. An anisaldehyde mixture (550 ml ethanol, 20.7 ml sulfuric acid, 6.3 ml acetic acid, and 15.3 ml 4-methoxy-benzaldehyde) or UV-light was used for detection. Flash chromatography was performed on TLC-grade silica gel 60H with eluting solvents indicated in the text.

Analytical GLC was performed with a Varian 3400 capillary GLC fitted

with a DB-Wax 30-m column. Preparative GLC was performed on a 6-m × 4-mm OV-351 column. The purity of the isolated final products was ≥99% except for compound **4**, for which the purity was only 88% (see Discussion). No sample contained any detectable amount of the natural pheromone component **1**. ^{1}H and ^{13}C NMR spectra were determined on a Varian XL-300 or 200 or on a Bruker DRX 400 spectrometer. High-resolution mass spectra were recorded on a Jeol JMS-SX 102 spectrometer. Infrared spectra were recorded on a Perkin Elmer 298. The synthetic schemes used for the preparation of **2–6** are shown in Figure 4. The synthesis for compounds **1** and **7–9** have previously been reported (Olsson et al., 1983; Liljefors et al., 1984; Hansson et al., 1996).

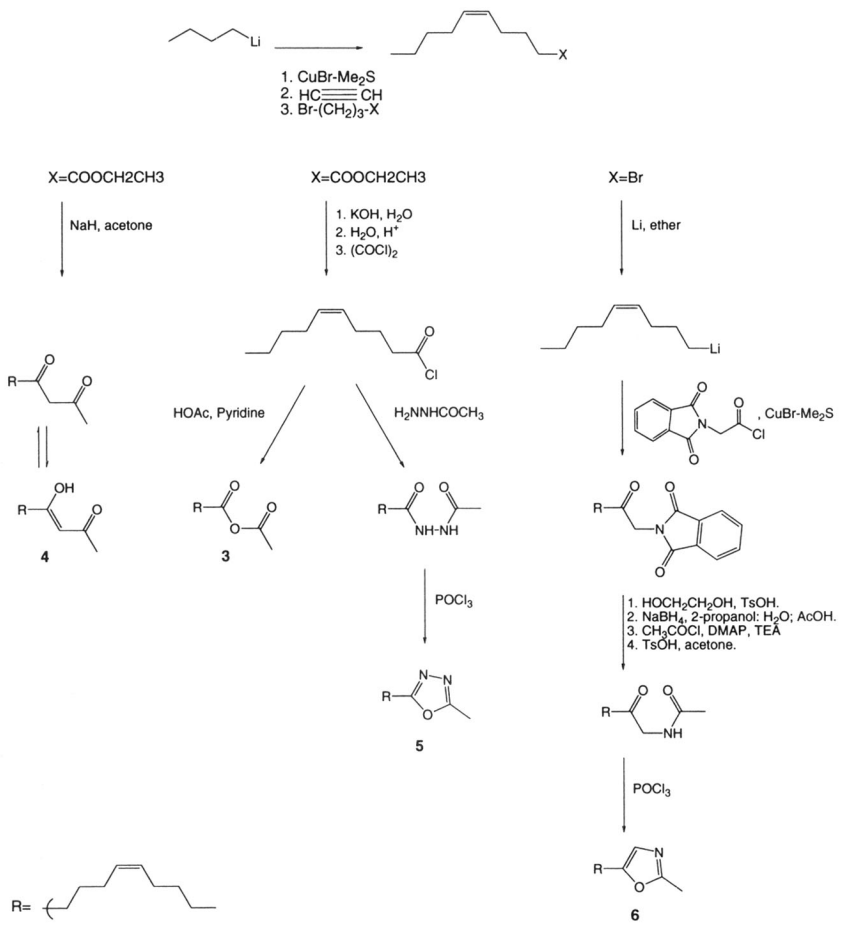

FIG. 4. Scheme for the preparation of analogs **2–6**.

Preparation of Lithium Bis[(Z)-hex-1-enyl] Cuprate. This compound was prepared by carbocupration of acetylene according to Gardette et al. (1985). To a well-stirred suspension of CuBr-Me_2S complex (8.2 g, 40 mmol) in ether (200 ml) at −50°C, 40.0 ml of 2.1 M butyl lithium was slowly added. The solution was allowed to reach −30°C and stirred for 30 min at this temperature. The almost clear solution was cooled to −50°C, and acetylene (2 liter, 80 mmol) was gently bubbled into the reaction mixture. The resulting solution was allowed to reach −25°C and then stirred for 30 min.

(Z)-5-Ethyl-decenoate (2) (X = $COOCH_2CH_3$ in Figure 4). To a stirred solution of lithium bis[(Z)-hex-1-enyl] cuprate (40 mmol) at −30°C, DMPU (14 ml) in 100 ml THF and ethyl-4-bromobutanoate (5.8 ml, 40 mmol) in 16 ml THF were successively added. The reaction mixture was slowly allowed to reach room temperature and stirred over night. After cooling to −20°C, the reaction was quenched with 5 M HCl (100 ml). After stirring for 15 min with air bubbling into the mixture, 100 ml pentane was added and the precipitate was filtered off with celite. The aqueous layer was extracted with ether, and the combined organic extracts were washed once with 17% NH_3, once with saturated NH_4Cl solution, and then dried over $MgSO_4$. The solvents were removed, and the crude product was purified by flash chromatography (pentane–ethyl acetate, 40:1). Compound **2** was afforded in two fractions: 3.5 g, 82% pure by GLC and 0.73 g, 70% pure by GLC. Total yield was 42%. The fraction to be analyzed and biologically tested was further purified by argentation chromatography (Houx et al., 1974), δ_H (300 MHz) 0.88 (t, 3H, $\mathbf{CH_3}$), 1.25(t, 3H, $\mathbf{CH_3}(CH_2O)$, 1.27–1.33 (m, 4H, $\mathbf{CH_2CH_2}$), 1.62–1.72 (m, 2H, $\mathbf{CH_2}$), 1.96–2.10 (m, 4H, $\mathbf{CH_2}CH{=}CH\mathbf{CH_2}$), 2.28 (t, 2H, $\mathbf{CH_2}CO$), 4.11 (q, 2H, $CH_3\mathbf{CH_2}O$), 5.26–5.44 (m, 2H, J = 10.9 Hz, $\mathbf{CH}{=}\mathbf{CH}$). δ_C (75.4 MHz) 14.0, 14.2, 22.3, 24.9, 26.5, 26.9, 31.9, 33.7, 60.2, 128.4, 131.0, 173.7. MS (high resolution) for $C_{12}H_{22}O_2$ calculated 198.1620, found 196.1618.

(Z)-5-Decenoic Acid. KOH (37 g) was dissolved in water (37 ml) and (Z)-5-ethyl-decenoate (**2**) (0.50 g, 2.6 mmol) was added. The reaction mixture was allowed to reflux over night. After cooling in an ice bath, the mixture was acidified with 5 M HCl, extracted with ether, and dried ($MgSO_4$). The solvent was removed, and the acid (0.43 g, 2.5 mmol) was isolated in 97% yield. δ_H (300 MHz) 0.88 (t, 3H, $\mathbf{CH_3}$), 1.24–1.35 (m, 4H, $\mathbf{CH_2CH_2}$), 1.62–1.74 (m, 2H, $\mathbf{CH_2}$), 1.97–2.12 (m, 4H, $\mathbf{CH_2}CH{=}CH\mathbf{CH_2}$), 2.36 (t, 2H, $\mathbf{CH_2}COOH$), 5.26–5.46 (m, 2H, $\mathbf{CH}{=}\mathbf{CH}$). IR: 3400–2500 (−OH), 1725–1690 (C=O).

(Z)-5-Decenoyl Chloride. (Z)-5-Decenoic acid (1 g, 5.9 mmol) was dissolved in dry ether and cooled to 0°C. Oxalyl chloride (3.0 ml, 16.2 mmol) and dimethyl formamide (60 μl, 0.9 mmol) were successively added. The reaction mixture was stirred for 30 min at 0°C and 3 hr at room temperature. The mixture was evaporated and CCl_4 was added, followed by further evaporation.

This procedure was repeated three times. The acid chloride was isolated in quantitative yield. δ_H (300 MHz) 0.88 (t, 3H, CH_3), 1.22–1.36 (m, 4H, CH_2CH_2), 1.71–1.80 (m, 2H, CH_2), 1.96–2.14 (m, 4H, $CH_2CH{=}CHCH_2$), 2.88 (t, 2H, CH_2COCl), 5.22–5.50 (m, 2H, CH=CH. IR: 1770–1830 (C=O).

1-Oxa-(Z)-5-Decenyl Acetate (3). Compound **3** was prepared according to Penn et al. (1993). (*Z*)-5-Decenoyl chloride (0.66 g, 3.5 mmol) in dry toluene (10 ml) was added to a solution of pyridine (0.29 ml, 3.5 mmol) and toluene. Stirring was continued until a white precipitate, the acylhalide–pyridinium complex, was formed (about 30 min). The reaction mixture was cooled to −10°C, and freshly distilled acetic acid (0.20 ml, 3.5 mmol) was added with stirring for 4 hr. The mixture was filtered through celite, rinsed with toluene, and the solvent was then removed. The residue (0.64 g) contained a mixture of the desired unsymmetric anhydride and the (*Z*)-5-decenoic acid anhydride. This mixture was distilled with a Kugel-Rohr apparatus, at approximately 3 mm Hg and 125°C. Great losses occurred and an 88% (NMR) pure product **3** was obtained. δ_H (300 MHz) 0.88 (t, 3H, CH_3), 1.22–1.35 (m, 4H, CH_2CH_2), 1.66–1.76 (m, 2H, CH_2), 1.97–2.14 (m, 4H, $CH_2CH{=}CHCH_2$), 2.20 (s, 3H, CH_3CO), 2.44 (t, 2H, CH_2CO, in unsym. anhydride) 2.43 (t, 4H, CH_2CO, in sym. anhydride); 88% versus 12%, 5.25–5.47 (m, 2H, J = 10.9 Hz, CH = CH). δ_C (75.4 MHz) 13.9, 22.2, 22.3, 24.1, 26.2, 26.9, 31.8, 34.5, 127.9, 131.6, 166.6, 169.3. IR: 1740–1760, 1800–1830 (C=O). MS (high resolution) for $C_{12}H_{20}O_3$ calculated 212.1412, found 212.1412.

*Preparation of (Z)-8-Tridecene,2,4-dione, 4-Hydroxy-(Z)-3,8-tridecadien-2-one (**4a, 4b**).* To a mixture of NaH (80% in mineral oil, 0.81 g, 27 mmol) and (*Z*)-5-ethyl decenoate, **2** (2.69 g, 13.5 mmol), freshly distilled acetone (2.0 ml, 27 mmol) was slowly added. When 30% of the acetone had been added, three drops of ethanol were added to start the reaction, followed by addition of the remaining acetone. The mixture was occasionally cooled on an ice bath to keep the temperature between 30 and 40°C. Dry ether (20 ml) was added, and the reaction mixture was stirred over night at room temperature. The unreacted NaH was destroyed by adding ethanol (1.5 ml), and, after stirring for 15 min, ice cooled acetic acid (1.7 ml, 30 mmol) and water (15 ml) were slowly added. The ether phase was separated, and the aqueous phase was extracted with ether. The combined organic extracts were washed three times with $NaHCO_3$ solution, once with water, and then dried ($CaSO_4$). The solvent was removed, and the residue was purified by flash chromatography (petroleum ether–ethyl acetate, 10:1) affording compound **4** (0.53 g, 2.5 mmol) in 19% yield. The amount to be biologically tested was further purified by flash chromatography (petroleum ether–ethyl acetate, 30:1), followed by preparative GLC. **4a**: δ_H (400 MHz) 0.91 (t, 3H, CH_3), 1.29–1.36 (m, 4H, $CHCH_2$), 1.64–1.72 (m, 2H, CH_2), 2.00–2.12 (m, 4H, $CH_2CH{=}CHCH_2$), 2.07 (s, 3H, CH_3CO), 2.29 [t, 3H,

$CH_2C(OH)=$], 5.30–5.46 (m, 2H, J = 10.8 Hz, CH=CH), 5.51 (s, 1H, [C(OH)=CHCO]. δ_C (100 MHz) 14.4, 22.8, 25.4, 26.1, 27.0, 27.4, 32.3, 38.1, 100.2, 128.9, 131.5, 191.9, 194.5. **4b**: δ_H (400 MHz) 2.25 (s, 3H, CH_3), 2.52 (t, 2H, CH_2CO), 3.58 (s, 2H, $COCH_2CO$). δ_C (100 MHz) 23.7, 26.7, 27.0, 31.3, 43.6, 58.4, 128.8, 131.7. Enol form to keto form ratio was 85:15 ($CDCl_3$). MS (high resolution) for $C_{13}H_{22}O_2$ calculated 210.1620, found 210.1621.

Acethydrazide. This compound was synthesized according to Henecka and Kurtz (1952). Hydrazine hydrate (15 ml, 0.3 mol) was heated to approximately 100°C, and ethyl acetate (22 ml, 0.22 mol) was added dropwise. The mixture was refluxed for two days and then evaporated. The residue was cooled, the crystals formed were treated with ether and chloroform (1:1), and the solvents were then removed. The crystalline acethydrazide (11.5 g, 0.15 mol, 68%) was allowed to dry, melting point 54–59°C (lit. 67°C). δ_H (300 MHz) 1.95 (s, 3H, CH_3CO), 3.85 (broad s, 2H, $NHNH_2$), 6.90 (broad s, 1H, $NHNH_2$).

N'-(Z)-5-Decenoic-N"-Acethydrazide. Acethydrazide (0.54 g, 7.3 mmol) was diluted in acetic acid (4 ml) and cooled on an ice bath. (Z)-5-Decenoyl chloride (100 g, 5.3 mmol) was slowly added (Brown et al., 1961). The mixture was stirred for 1 hr at room temperature, poured into water, and neutralized with $NaHCO_3$ solution. The aqueous solution was extracted with ether (three times), and the combined organic extracts were washed twice with K_2CO_3 solution, once with water, and then dried (Na_2CO_3). The solvent was removed, and the resultant crystals (0.70 g, 3.1 mmol, 58%) had a melting point of 75–77°C after recrystallization in petroleum ether (60/70). δ_H (300 MHz) 0.86 (t, 3H, CH_3), 1.21–1.34 (m, 4H, CH_2CH_2), 1.62–1.73 (m, 2H, CH_2), 1.94–2.08 (m, 4H, $CH_2CH=CHCH_2$), 2.03 (m, 3H, CH_3CONH), 2.26 (t, 2H, CH_2CONH), 5.23–5.42 (m, 2H, CH=CH), 9.45 (broad s, 1H, NH), 9.77 (broad s, 1H, NH).

2-Methyl-5-[(Z)-4-Nonenyl]-oxadiazole (5). The hydrazide (100 mg, 0.44 mmol) was treated with $POCl_3$ (0.5 ml) and heated to reflux. After 3 hr the mixture was quenched with water, made basic with K_2CO_3 solution, and then extracted with ether. The combined ether extracts were dried ($Na_2CO_3/MgSO_4$), and the solvent was removed. The residue was purified by flash chromatography (ethyl acetate), and compound **5** was obtained (60 mg, 0.29 mmol) in 64% yield. The compound was further purified by preparative GLC. δ_H (400 MHz) 0.90 (t, 3H, CH_3), 1.30–1.36 (m, 4H, CH_2CH_2), 1.81–1.89 (m, 2H, CH_2), 2.00–2.05 (m, 2H, $=CHCH_2$), 2.16 (m, 2H, $=CHCH_2$), 2.51 [s, 3H, $CH_3C(O)=N$], 2.82 [t, 2H, $CH_2C(O)=N$], 5.31–5.48 (m, 2H, J = 10.8 Hz, CH=CH). δ_C (100 MHz) 11.4, 14.4, 22.8, 25.2, 26.84, 26.85, 27.4, 32.3, 128.2, 132.0, 164.0, 167.5. High-resolution CI mass spectrum for $C_{12}H_{21}N_2O$ ($M+H^+$), calculated 209.1654, found 209.1654.

1-Bromo-(Z)-4-nonene. This compound (X = Br in Figure 4) was synthesized in the same manner as compound **2** from lithium bis[(*Z*)-hex-1-enyl] cuprate (0.08 mol) and 1,3-dibromopropane (20 ml, 0.20 mol) (Gardette et al., 1983). The crude product was distilled and the halide (9.0 g, 0.044 mol) was obtained at 90–100°C/12 mm Hg and in 55% yield. δ_H (300 MHz) 0.90 (t, 3H, CH_3), 1.25–1.38 (m, 4H, CH_2CH_2), 1.86–2.24 (m, 6H, $CH_2CH{=}CHCH_2CH_2$), 3.42 (t, 2H, CH_2Br), 5.26–5.49 (m, 2H, J = 10.8 Hz, $CH{=}CH$).

1-Lithium-(Z)-4-nonene. Lithium (30% in mineral oil, 0.81 g, 34.7 mmol) and ether (4 ml) were cooled to −10°C, and 1-bromo-(*Z*)-4-nonene (3.00 g, 14.6 mmol) in ether (14 ml) was slowly added. After the addition was complete, the reaction mixture was stirred for 1 hr at 0°C. The 1-lithium-(*Z*)-4-nonene was titrated according to Watson and Eastham (1967) and found to be 0.5 M, 71% yield.

2-(2-Ethanoic acid)-isoindole-1,3-dione. This compound was prepared according to Billman and Harting (1948). Phthalic anhydride (20.0 g, 0.135 mol) and glycine (10.0 g, 0.133 mol) were placed in a large sublimation apparatus and heated to 180°C. The phthalic anhydride that sublimed on the cold finger was pushed down into the reaction mixture three times during 15 min. Thereafter, the excess of phthalic anhydride was allowed to sublime on the cold finger, and the residue was recrystallized from ethanol and water (9:1). The phthalimide-protected acid (25.3 g, 0.123 mol) was isolated in 92% yield. δ_H (200 MHz) 4.50 (s, 2H, CH_2), 7.70–7.78 (m, 2H, Ar**H**), 7.85–7.92 (m, 2H, Ar**H**).

2-(2-Ethanoyl chloride)-isoindole-1,3-dione. The acid halide was prepared as described above for (*Z*)-5-decenoyl chloride from phthalimide ethanoic acid (0.53 g, 2.6 mmol) in ether (40 ml) and by the addition of oxalyl chloride (1.3 ml, 7.0 mmol) and dimethyl formamide (26 ml). The product was isolated in quantitative yield. δ_H (200 MHz) 4.82 (s, 2H, CH_2), 7.75–7.82 (m, 2H, Ar**H**), 7.88–7.95 (m, 2H, Ar**H**).

2-[2-Oxa-(Z)-6-undecenyl]-isoindole-1,3-dione. This compound was synthesized according to Posner (1975). 1-Lithium-(*Z*)-4-nonene (10.0 mmol) was slowly added to a cooled (−40°C) suspension of CuBr–Me_2S (1.00 g, 4.8 mmol) and ether (20 ml). The mixture was allowed to reach −20°C and stirred for 0.5 hr. After cooling to −65°C, phthalimide ethanoyl chloride (2.40 g, 10.7 mmol) in ether (100 ml) was added. The reaction mixture was stirred for 1 hr, then quenched with water, and allowed to reach room temperature. The organic phase was separated, and the aqueous phase was extracted with ether. The combined organic extracts were washed with saturated NH_4Cl and dried ($MgSO_4$). After evaporation, the crude product was purified by flash chromatography (heptane–ether; 4:1). The product (0.59 g, 1.9 mmol) was obtained in 40% yield. δ_H (300 MHz) 0.88 (t, 3H, CH_3), 1.24–1.36 (m, 4H, CH_2CH_2),

1.66–1.75 (m, 2H, CH_2), 1.96–2.12 (m, 4H, $CH_2CH{=}CHCH_2$), 2.52 (t, 2H, CH_2CO), 5.25–5.46 (m, 2H, J = 10.8 Hz, CH=CH), 7.70–7.78 (m, 2H, ArH), 7.82–7.90 (m, 2H, ArH).

2-{2-[(Z)-4-Nonenyl]-[1,3]-dioxolan-2-yl methyl}-isoindole-1,3-dione. The β-keto function was protected as an acetal by refluxing the compound (540 mg, 1.72 mmol) with ethylene glycol (1.2 ml, 2.1 mmol) and *p*-toluene sulfonic acid monohydrate (20 mg, 0.10 mmol) in benzene (100 ml) with a Dean-Stark apparatus over night. After cooling to room temperature, ether was added, and the organic phase was washed with $NaHCO_3$ solution and brine and dried ($NaSO_4$). The solvents were removed, and the product (550 mg, 1.54 mmol) was isolated in 90% yield. δ_H (300 MHz) 0.88 (t, 3H, CH_3), 1.22–1.37 (m, 4H, CH_2CH_2), 1.42–1.55 (m, 2H, CH_2), 1.60–1.68 (m, 2H, CH_2), 1.95–2.10 (m, 4H, $CH_2CH{=}CHCH_2$), 3.80 (s, 2H, CH_2N), 3.90–4.15 (m, 4H, OCH_2CH_2O), 5.28–5.40 (m, 2H, CH=CH), 7.68–7.76 (m, 2H, ArH), 7.82–7.90 (m, 2H, ArH).

{2-[(Z)-4-Nonenyl]-[1,3]dioxolan-2-yl methyl}-amine. The phthalimide protecting group was removed by a method developed by Osby et al. (1984). The compound (500 mg, 1.40 mmol) was dissolved in 2-propanol (14 ml) and water (3.2 ml). $NaBH_4$ (0.55 g, 14.5 mmol) was added in small portions, and the reaction was allowed to stir over night. Then acetic acid (5 ml) was carefully added. When foaming had subsided, the mixture was heated to 80°C for 2 hr. After cooling to room temperature, it was made basic with Na_2CO_3 and then extracted with ether. The organic phase was dried (Na_2SO_4), and the solvents were removed. The crude amine was purified by flash chromatography (ethyl acetate–triethyl amine, 4:1), and the pure product (210 mg, 0.92 mmol) was isolated in 66% yield. δ_H (300 MHz) 0.88 (t, 3H, CH_3), 1.22–1.68 (m, 8H, CH_2), 1.96–2.10 (m, 4H, $CH_2CH{=}CHCH_2$), 2.72 (s, 2H, CH_2N), 3.96 (s, 4H, OCH_2CH_2O), 5.28–5.42 (m, 2H, CH=CH).

N-{2-[(Z)-4-Nonenyl]-[1,3]-dioxolan-2-yl methyl}-acetamide. The amine (200 mg, 0.88 mmol), 4-dimethylaminopyridine (10 mg, 0.08 mmol), and triethyl amine (0.5 ml) in dichloromethane (10 ml) were cooled on an ice bath, and acetic anhydride (0.15 ml, 1.60 mmol) was added. The reaction was allowed to reach room temperature and stirred for 6 hr. It was then poured onto ice water and extracted with ether. The organic phase was washed with brine, dried ($NaSO_4$), and concentrated. The crude amide was purified by flash chromatography (ethyl acetate), and the product (210 mg, 0.78 mmol) was obtained in 89% yield. δ_H (300 MHz) 0.88 (t, 3H, CH_3), 1.22–1.48 (m, 6H, CH_2), 1.57–1.66 (m, 2H, CH_2), 1.94–2.08 (m, 4H, $CH_2{=}CHCH_2$), 2.00 (s, 3H, CH_3CO), 3.39 (d, 2H, CH_2NH), 3.95 (s, 4H, OCH_2CH_2O), 5.25–5.40 (m, 2H, CH=CH), 5.60 (broad s, 1H, NH).

N-[2-Oxo-(Z)-6-undecenyl]-acetamide. In order to remove the acetal, the amide (200 mg, 0.74 mmol) was refluxed in acetone (50 ml) with *p*-toluene

sulfonic acid monohydrate (20 mg, 0.10 mmol) over night. After cooling, ether was added, and the organic phase was washed with saturated $NaHCO_3$ solution and brine, dried ($NaSO_4$), and concentrated. The product (135 mg, 0.60 mmol) was obtained in 81% yield. δ_H (300 MHz) 0.89 (t, 3H, CH_3), 1.24–1.38 (m, 4H, CH_2CH_2), 1.62–1.74 (m, 2H, CH_2), 1.94–2.10 (m, 4H, $CH_2CH{=}CHCH_2$), 2.02 (s, 3H, CH_3CO), 2.45 (t, 2H, CH_2CO), 4.13 (d, 2H, CH_2NH), 5.23–5.46 (m, 2H, J = 10.8 Hz, $CH{=}CH$), 6.24 (broad s, 1H, NH).

2-Methyl-5-[(Z)-4-nonenyl]-oxazole (6). Compound **6** was synthesized by treating the β-keto amide (120 mg, 0.53 mmol) with $POCl_3$ in the same manner as in the synthesis of compound **5.** The crude product was purified by flash chromatography (ethyl acetate), and the oxazole (60 mg, 0.29 mmol) was isolated in 53% yield. The oxazole was further purified by preparative GLC. δ_H (400 MHz), 0.90 (t, 3H, CH_3), 1.30–1.36 (m, 4H, CH_2CH_2), 1.65–1.73 (m, 2H, CH_2), 1.99–2.14 (m, 4H, $CH_2CH{=}CHCH_2$), 2.41 [s, 3H, $CH_3C(O){=}N$], 2.61 [t, 2H, $CH_2(CO){=}N$], 5.32–5.46 (m, 2H, J = 10.8 Hz, $CH{=}CH$), 6.59 [s, 1H, $CH(N){=}C$]. δ_C (100 MHz) 14.37, 14.41, 22.8, 25.4, 26.9, 27.4, 28.0, 32.3, 122.4, 128.9, 131.4, 152.8, 160.6. High-resolution CI mass spectrum for $C_{13}H_{22}ON$ (MH^+), calculated 208.1701, found 208.1702.

Electrophysiology. The activities of compounds **1–9** were determined by single-cell electrophysiology (Kaissling, 1974). The olfactory receptor-cell specifically tuned to (*Z*)-5-decenyl acetate, present in the antennal sensilla type SW1 of *Agrotis segetum*, was used (Hallberg, 1981; Löfstedt et al., 1982; van der Pers and Löfstedt, 1983). The method was modified according to van der Pers and den Otter (1978) and has been previously described (Liljefors et al., 1987; Bengtsson et al., 1990).

The stimulus amounts used for pheromone component **1** were 10^{-4}–10^{-1} μg and for analogs **2–6**, 10^{-1}–10^{2} μg in decadic steps. For each loading, 10 replicates were recorded and the mean value of the action potentials generated during 1 sec from the onset of stimulation was used in the construction of the dose–response curves. The errors were expressed as standard errors of the mean (SEM). The electrophysiological activity of each compound in relation to **1** is expressed as the reciprocal of the relative quantities required to elicit the same response from the receptor as the natural pheromone component **1**.

Differences in volatilities for compounds **2**, **3**, and **4** were taken into account by correcting the activities by using relative vapor pressures as previously described (Liljefors et al., 1985; Bengtsson et al., 1990; Gustavsson et al., 1995). The correction added to the logarithm of the relative activity for compound **2** is based on vapor pressure data (Dykyj and Répaš, 1979) for propyl acetate, ethyl propanoate, butyl acetate, and ethyl butanoate and was −0.06. The correction for compound **3** is based on vapor pressures for acetic anhydride and ethyl acetate and was 1.26. The correction added for compound **4** was 1.01,

based on data for acetyl acetone and ethyl acetate. The correction used for **6** was based on vapor pressure data for oxazole (DIPPR Database, American Institute of Chemical Engineers) and for methyl formate (Dykyj and Répaš, 1979) and was 0.64. As no vapor pressure data could be found for oxadiazole, this correction was also employed for compound **5**. For compounds **7–9** the uncorrected activities, taken from Hansson et al. (1996), were used.

Quantum Mechanical Calculations. Electrostatic potentials for the polar functional groups in compounds **1–9** (R = CH_3 in Figure 3) were calculated using quantum mechanical *ab initio* calculations. The electrostatic potential V at a point r is defined by equation 1 (Politzer and Murray, 1991):

$$V(r) = \sum_{\alpha=1}^{N} \frac{Z_\alpha}{|R_\alpha - r|} - \int \frac{\rho(r')}{|r' - r|} dr' \quad (1)$$

where N is the number of nuclei, Z_α is the charge of the αth nucleus located at R_α, and $\rho(r)$ is the electron density function of the molecule. Integration is carried out over all space. The electrostatic potential at point r is calculated by letting a unit positive charge at that point interact with the nuclei and electrons of the molecule. Thus, an electrophile will be attracted to negative regions of $V(r)$, most strongly to the point where $V(r)$ has its most negative value.

For the calculations of the electrostatic potentials, the HF/6-31G* basis set was employed with geometries calculated by using the HF/3-21G(*) basis set. For compounds **3**, **4**, and **7**, additional conformational analysis were performed using the 6-31G* basis set. The calculations were performed on a Silicon Graphic Indy Workstation using the Spartan computer program, (Wavefunction Inc.).

RESULTS

Chemicals. In order to obtain the *Z* double bond, a selective carbocupration of acetylene, a method developed by Gardette et al. (1985), was used. This method has previously been employed for the syntheses of a number of (*Z*)-5-decenyl acetate analogs (Jönsson et al., 1991a,b, 1992, 1993; Gustavsson et al., 1995). The anhydride, compound **3**, was synthesized from the acylpyridinium complex and acetic acid according to Penn et al. (1993). However, the anhydride could not be satisfactorily purified by distillation with Kugel-Rohr apparatus (due to the small amounts available, an ordinary distillation could not be undertaken). The final product obtained was a mixture, which, according to NMR spectroscopy, contains 88% of the desired unsymmetric anhydride **3** and 12% of the symmetric (*Z*)-5-decenoic acid anhydride. As the vapor pressure of this compound should be much lower than that of **3**, this symmetric anhydride should not significantly influence the result of the electrophysiological testing. Compound **4** was synthesized *via* a Claisen acylation according to Swamer and

Hauser (1950). To obtain the heterocyclic compounds **5** and **6**, cyclization of the hydrazide compound and the β-keto amide, respectively, was performed with the dehydrating reagent phosphorus oxychloride according to Hayes et al. (1955).

Molecular Electrostatic Potentials. The electrostatic potentials for the functional groups of compounds **1–9** (Figure 3, R = CH_3) were calculated using conformations in which all heavy atoms are located in the same plane, corresponding to the conformation of the acetate group in the proposed bioactive conformation of **1** (Figure 1). For all analogs, except compounds **3**, **4b**, and **7**, the planar conformation is the lowest energy conformation found by the *ab initio* calculations. The conformation of acetic anhydride corresponding to the polar functional group in compound **3** is, according to gas-phase electron-diffraction experiments, a twisted structure with a dihedral angle between the two carbonyl groups of 79° (Vledder et al., 1971). Energy minimizations, using the 6-31G* basis set, gave two geometries of low conformational energies, one with a dihedral angle between the two carbonyl functions of 51° and one with 90°, which is 0.4 kcal/mol less stable. The experimental value is a mean value of the interchange between the two low-energy conformations. The planar *cis* conformation used in the calculations of electrostatic potentials is computed to be 1.1 kcal/mol higher in energy than the most stable conformation.

Compound **4** may exist in two tautomeric forms, **4a** and **4b** (Figure 3). Experimental data (Iijima et al., 1987) show that the enol form **4a** predominates in the gas phase (ca. 98% enol form). However, the keto–enol equilibrium is sensitive to the polarity of the environment. Thus, in water, the keto form is the dominating tautomer (84%) (Emsley, 1984). In our calculations (6-31G* basis set), the most stable keto form is found to be twisted with an angle of 96° between the carbonyl groups. This is in agreement with experimental results. Infrared and Raman spectroscopy give an angle of 90° (Ernstbrunner, 1970; Buemi and Gandolfo, 1989), while electron diffraction gives a corresponding angle of 49° (Lowery et al., 1971). The planar *cis* conformation was found to be as much as 6.6 kcal/mol less stable than the twisted conformation.

For compound **7**, the calculated lowest energy conformation, using the 6-31G* basis set, was found to be a structure with a C-S-C-C dihedral angle of 80°. However, the planar conformation used in the calculations of electrostatic potentials is only 0.3 kcal/mol higher in energy.

The calculated electrostatic potentials are shown in Figure 5. The potentials are displayed as contours in the plane of the heavy atoms of the various functional groups. The local minima, i.e., the points of maximum negative electrostatic potentials, are indicated by arrows, and the interaction energies (in kilocalories per mole) with a unit positive charge in these points are shown.

In the case of the acetate group (**1**), there are three regions of strong negative electrostatic potentials, two in the vicinity of the carbonyl oxygen in

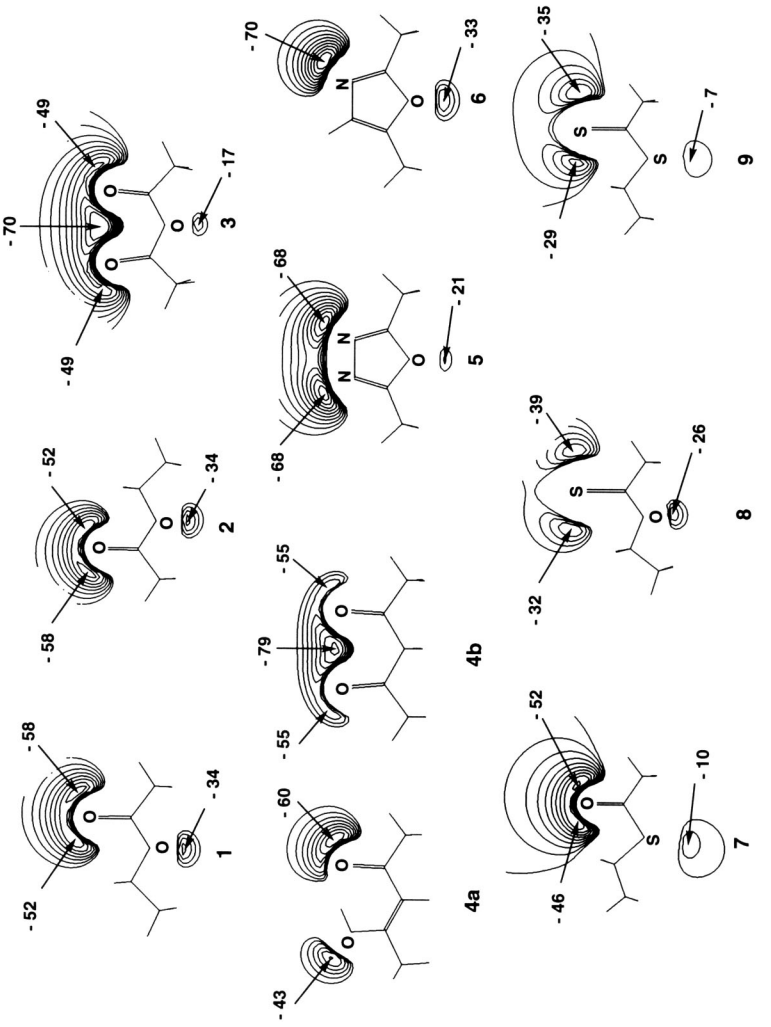

FIG. 5. Molecular electrostatic potentials in the plane of the polar functional groups of compounds **1–9** calculated by *ab initio* HF/6-31G*//HF/3-21G(*) calculations. Interaction energies are in kilocalories per mole.

the directions of the oxygen lone pairs and one located around the ether oxygen. These regions correspond to regions A, B, and C in Figure 2.

The sulfur analogs **7–9** display qualitatively the same regions of negative electrostatic potentials as for **1**, but the exchange of oxygen by sulfur significantly decreases the sizes of the interaction energies. As shown by compounds **7** and **8**, the electrostatic potentials, compared to those of **1**, are not only affected in the areas in the vicinity of the exchanged atom but also in other areas. For compound **7**, an exchange of the oxygen in the ether position by a sulfur decreases not only the size of the maximum interaction energy in area C, but also lowers the energies of attraction in areas A and B. For compound **8**, the exchange of a carbonyl group for a thiocarbonyl group affects not only areas A and B but also C.

The "reversed" ester group **2** has areas A and C in common with the acetate group (**1**) but introduces a new area of strong negative electrostatic potentials, D, which does not have a correspondence in **1**. The anhydride **3**, the enol and keto forms of **4** (**4a** and **4b**), and the oxadiazole **5** also display strong negative electrostatic potentials in this area (D). It should be noted that the enol form **4a** only has area B in common with the acetate (**1**). The oxazole **6** has only two regions of strongly negative electrostatic potentials corresponding to areas B and C in **1**.

In summary, the electrostatic potentials of the sulfur analogs **7–9** mimic the electrostatic potentials in areas A, B, and C of **1**. The reversed ester **2** mimics **1** with respect to areas A and C and introduces area D. The anhydride **3** and the oxadiazole **5** display similarities with **1** in areas A, B, and C, and **4b** mimics **1** in areas A and B. The enol form of **4** (**4a**) has only a strong negative electrostatic potential in area B in common with **1**. Furthermore, **3–5** have negative electrostatic potentials in an area D which is not present in **1**. Finally, the oxazole **6** mimics **1** with respect to the electrostatic potentials in areas B and C.

Receptor Cell Responses. Relative single-cell activities for compounds **1–9** are shown in Figure 6. The activities for compounds **1–6** are corrected for differences in volatility (see Methods and Materials). The data for **7–9** are taken from Hansson et al. (1996). Due to lack of vapor pressure data, the activities for these analogs are uncorrected. Since these compounds should be less volatile than **1**, the activities for **7–9**, shown in Figure 6, may be somewhat underestimated.

The most active analog is the thiocarbonyl analog **8**, which is found to be 90 times less active than **1**. The thioether analog **7** also displays significant activity, while the dithio analog **9** is much less active. Interestingly, the oxazole analog **6** displays moderate activity, whereas the structurally similar oxadiazole analog **5** is essentially inactive. Compound **4** (enol form is assumed, see Dis-

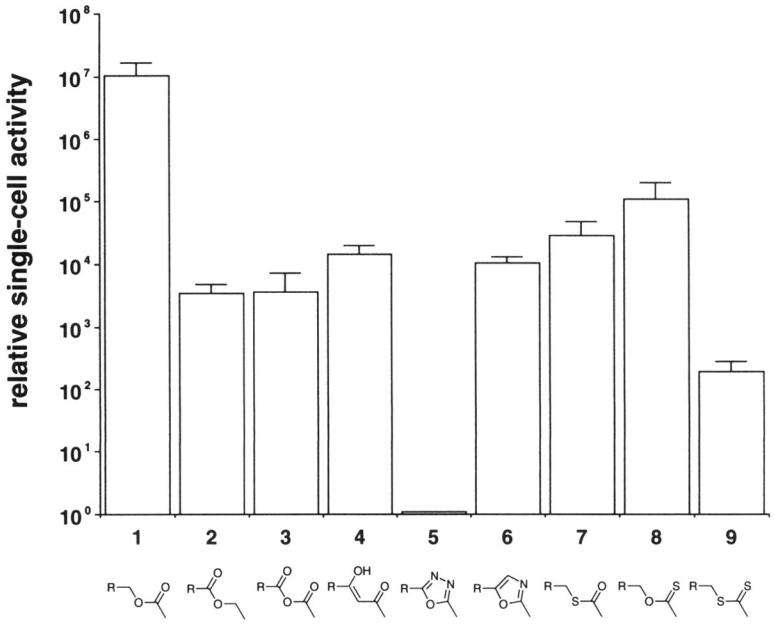

FIG. 6. Relative electrophysiological single-cell activities (+SEM) for compounds **1–9**. The activities for compounds **1–6** are corrected for differences in volatility. The data for **7–9** are taken from Hansson et al. (1996).

cussion) shows a somewhat higher activity than the equally active analogs **2** and **3**.

DISCUSSION

It is reasonable to assume that the acetate group of the natural pheromone component **1** is interacting with the receptor *via* hydrogen bonding involving one or both oxygen atoms. This type of interaction has previously been proposed for the binding of pheromone components to pheromone binding proteins (Prestwich et al., 1995; Prestwich, 1996). In a study of the effects on the activity of modifications of the acetate group of (Z)-7-dodecenyl acetate (Liljefors et al., 1984), it was shown that the removal of the carbonyl group results in a virtually inactive compound. The substitution of the ether oxygen by a CH_2 group decreases the activity by a factor of 100.

Various combinations of hydrogen bond interactions and hydrogen bond directions are possible for the acetate group in **1**. These possibilities correspond

to maximum negative electrostatic potentials in the plane of the acetate group. The electrostatic potential has previously been successfully used to predict the sites and directionality of hydrogen bonds in a variety of systems. In addition, good correlation has been found between calculated hydrogen bond energies and the value of the electrostatic potential in appropriate areas (Politzer and Murray, 1991).

The calculated electrostatic potentials indicate three areas (A, B, and C) of possible hydrogen bond interactions between **1** and hydrogen-bond-donating amino acid residues in the receptor. The calculated electrostatic potentials of compounds **1–9** may, in conjunction with their single-cell activities, be employed to draw conclusions about the most probable sites and directions of the assumed hydrogen bond interactions.

Sulfur is a weaker hydrogen bond acceptor than oxygen (Chang et al., 1991; Hansson et al., 1996), which is reflected in the smaller size of the maximum negative electrostatic potentials in the vicinity of the sulfur atom. Thus, the reduced activities of the sulfur analogs **7–9** may be understood in terms of weaker hydrogen bond interactions with the receptor. Compounds **1** and **7** have similar electrostatic potentials in the vicinity of the carbonyl group. However, in contrast to the relatively strong potential caused by the ether oxygen in **1**, the corresponding thioether in **7** only gives rise to a weak electrostatic potential. This difference rationalizes the lower activity of **7**, which, furthermore, implies both ester oxygens in **1** are contributing to the interaction between the acetate group and the receptor. The same conclusion may be drawn by comparing the activities and the calculated electrostatic potentials of compounds **8** and **9**. These compounds have the same magnitude of the electrostatic potential in the thiocarbonyl areas A and B, but have significantly different values in area C where **9** displays a much less negative potential than **8**. This difference shows up in a lower activity of **9** by a factor of about 600. These results are in line with previously reported results for the interaction between (*Z*)-7-dodecenyl acetate and its receptor (Liljefors et al., 1984).

Analogs **7** and **9** have similar electrostatic potentials in areas C, but **7** has a much stronger ability to interact *via* a hydrogen bond to the carbonyl group (areas A and B). This is reflected in the significantly higher activity of **7** compared to that of **9**. Compound **9** has weak electrostatic potentials in each of the three areas A, B, and C, and has consequently the lowest activity of the sulfur analogs.

Compound **2** is 2800 times less active than **1**. The most notable feature of the electrostatic potential of **2** is that there is no local potential minimum in **2** corresponding to area B in compound **1**, but instead there is a local minimum D that does not have a correspondence to **1**. The strongly reduced activity of **2** is most probably not due to the presence of area D for the following reasons. Compound **4**, which is somewhat more active than **2** also has a strongly negative

electrostatic potential in area D in the enol (**4a**) as well as in the keto form (**4b**). However, the decreased activity of **4** compared to that of **1** is clearly due to the lack of a negative electrostatic potential corresponding to that in the vicinity of the ether oxygen in **1** (area C). For this reason, an activity of **4** somewhat lower than that of **7**, which has a weak electrostatic potential in area C, is expected. This is also observed. Thus, the low activity of **2** can probably be attributed to the lack of a negative electrostatic potential in area B.

The lowest energy conformation on the diketo group in **4b** is highly twisted. Such a comformation is not compatible with a bioactive conformation corresponding to that of **1** in Figure 1. A more or less planar conformation is required for **4b** to fit the model. However, this type of conformation has a very high conformational energy. The planar conformation is calculated to be 6.6 kcal/mol higher than the lowest energy one (see above). This high energy makes it less probable that **4** interacts with the receptor in its keto form **4b**. The more probable enol form (**4a**) only displays two areas of large negative electrostatic potentials corresponding to areas B in Figure 2 and D as defined above. According to the discussion above, the activity decrease of **4b** compared to **1** is completely rationalized by the lack of a negative electrostatic potential in area C. Thus, as **4a** lacks an area of negative electrostatic potential corresponding to area A of **1**, it may be concluded that area A is of less importance for the interaction of **1** with its receptor.

The anhydride analog **3** displays a weak electrostatic potential in area C but a potential of the same magnitude as that of **7** in area B and a very strong one in area A. In addition, **3** also has a strong negative potential in area D. The decrease in activity of **3** by a factor of 2800 compared to that of **1** indicates that the presence of a very strong negative electrostatic potential in area A may negatively influence the activity. However, it should be noted that the anhydride **3** is easily hydrolyzed, which may affect the amount of compound that is actually reaching the receptor resulting in a lower than expected activity.

According to the discussion above, the oxazole gorup of compound **6** should be a good bioisostere to the acetate group of **1**. The activity of this compound supports the conclusion that a negative electrostatic potential in area B is crucial for the activity. A lack of a strong negative electrostatic potential in area A is of minor importance for the activity, and the decreased activity of **6** compared to **1** is most probably due to repulsive steric interactions between the hydrogen of the oxazole ring and the receptor. Such a steric repulsion may also be the reason why **8** displays a somewhat higher activity than **6**, despite the fact that the electrostatic potentials in areas B and C are more negative in **6** than in **8**.

In view of the moderate activity of **6**, the essential inactivity of compound **5** is unexpected and difficult to rationalize. However, the strongly negative electrostatic potential in the area between areas A and D in Figure 5 is unique

among the present analogs with respect to its position as well as its directional properties and may be detrimental to the activity of this compound.

CONCLUSIONS

None of the modifications of **1** studied above result in a fully active bioisosteric replacement of the acetate group in **1**. However, by analyzing the electrophysiological single-cell activities in terms of the molecular electrostatic potentials of the polar functional groups, as calculated by *ab initio* quantum mechanical calculations, it may be concluded that both oxygens of the acetate group in **1** contribute to the interaction between the pheromone component and its receptor. Furthermore, the results strongly indicate that the crucial interaction between the carbonyl group and the receptor, which is most probably a hydrogen bonding interaction, takes place in a direction pointing away from the hydrocarbon chain of the pheromone component, i.e., in the direction of the maximum negative electrostatic potential in area B in Figure 2.

The calculated weaker electrostatic potentials in the vicinity of a sulfur atom compared to those at the corresponding positions about an oxygen atom rationalize the lower activity of the sulfur analogs. The replacement of the acetate group in **1** by an oxazole ring system gives a pheromone component analog with a moderate electrophysiological activity. However, the corresponding replacement by an oxadiazole ring leads to a virtually inactive compound. Thus, the pattern of the electrostatic potential about the oxazole ring is compatible with a productive interaction with the receptor, whereas that about the oxadiazole ring is not.

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