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STRUCTURE–ACTIVITY RELATIONSHIPS FOR CHAIN-SHORTENED ANALOGS OF (Z)-5-DECENYL ACETATE, A PHEROMONE COMPONENT OF THE TURNIP MOTH, *Agrotis segetum*[1]

MARIE BENGTSSON,[2] TOMMY LILJEFORS,[2] BILL S. HANSSON,[3] CHRISTER LÖFSTEDT,[3] and SYLVIA V. COPAJA[3,4]

[2]*Department of Organic Chemistry 3*
Chemical Center, University of Lund
P.O. Box 124, S-221 00 Lund, Sweden

[3]*Department of Animal Ecology*
Ecology Building, University of Lund
S-223 62 Lund, Sweden

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Abstract—Structure–activity relationships for chain-shortened analogs of (Z)-5-decenyl acetate, a pheromone component of the turnip moth, *Agrotis segetum*, have been studied by electrophysiological single-sensillum technique and interpreted in terms of a previously reported receptor–interaction model. The results indicate that the terminal methyl group, as well as the acetate group, interacts with highly complementary receptor sites. The terminal alkyl chain is suggested to interact with a hydrophobic "pocket" extending over the two methylene groups closest to the terminal methyl group. The amounts of stimulus actually released from the odor source have been studied. The results demonstrate the necessity to take differences of volatility into account in comparisons of electrophysiological data for compounds of different chain lengths. It is shown that relative vapor pressures may to a good approximation be employed to estimate correction factors.

Key Words—Structure–activity, single-cell recordings, receptor interaction, (Z)-5-decenyl acetate, chain-shortened analogs, volatility, vapor pressure, *Agrotis segetum*, Lepidoptera, Noctuidae.

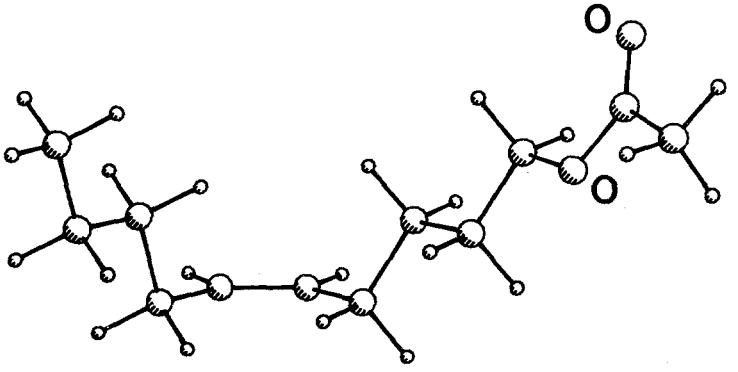
[1] Schiff., Lepidoptera: Noctuidae.
[4] Present address: Department of Chemistry, University of Chile, Casilla 653, Santiago, Chile.

INTRODUCTION

In a series of papers, we have reported the development and applications of a model for the interaction between a monoolefinic pheromone component and its receptor, employing structures and conformational energies calculated by the molecular mechanics method (Liljefors et al., 1984, 1985, 1987; Bengtsson et al., 1987). This model has been successfully used to rationalize the effects of chain elongation (Liljefors et al., 1985, 1987), change of double-bond configuration (Liljefors et al., 1987), and the introduction of an additional (*E*)-double bond at various positions (Bengtsson et al., 1987) on electrophysiological single-cell activities of analogs of (Z)-5-decenyl acetate (**1**), a sex pheromone component of the turnip moth, *Agrotis segetum* (Bestmann et al., 1978; Arn et al., 1980; Löfstedt et al., 1982).

Furthermore, the receptor–interaction model has been employed to predict the biologically active conformation of (Z)-5-decenyl acetate (Bengtsson et al., 1987). The predicted conformation is shown in Scheme 1. This prediction has recently been corroborated by the use of conformationally constrained analogs (Bengtsson, 1988).

The basic idea in our previous structure–activity studies is that, in order to show activity, it is necessary for an analog to mimic the natural pheromone component with respect to the spatial locations of the acetate group, the double-bond, and the terminal methyl group. It is assumed that the receptor cavity contains highly complementary interaction sites for these three molecular parts. All analogs previously studied were chosen for their ability to mimic the natural substrate in this respect through conformational rearrangements. We found that the observed electrophysiological activities for such analogs were determined essentially by the conformational energies required for the analogs to mimic the natural substrate.



SCHEME 1.

Since our receptor–interaction model at the present stage of its development requires mimicking of the acetate group, the double bond, and the terminal methyl group of the natural substrate, it cannot be applied to compounds that are unable to mimic the natural substrate in this respect. Thus, an important class of analogs that presently cannot be treated by our model is chain-shortened analogs. The acetate group, the double bond, and the terminal methyl group in this type of compound cannot simultaneously interact optimally with all three receptor sites of our model.

Chain shortening has consistently been found to lead to a decrease in the activity. However, for analogs one or two methylene units shorter than the natural component, a significant amount of the activity seems to be retained (Priesner et al., 1975; Priesner, 1979a,b, 1983; Struble and Byers, 1987).

Most of the previous studies on chain-shortened analogs have been performed with the EAG technique or by field-trapping experiments. Since chain-shortened analogs may be structurally very close to more than one of the components in the pheromone blend of the studied species, they may interact with more than one receptor type. Thus, the results of such studies are not suitable to be directly interpreted in terms of interactions with a single receptor type. In this respect, only single-cell recordings can provide unambiguous experimental data.

In order to further develop our receptor-interaction model, we have in the present paper studied a series of chain-shortened analogs, compounds **2–7**, of (*Z*)-5-decenyl acetate **1** (Figure 1). We report the synthesis of and electrophysiological single-cell measurements on them. The implications of the experimental data on the shape and other properties of the receptor cavity are discussed.

To corroborate the results, the accuracy of the stimulation system was investigated with respect to differences in volatility between test compounds and different amounts loaded onto the odor source.

METHODS AND MATERIALS

Chemicals. Final products were purified by flash chromatography (Taber, 1982a,b) on TLC-Silica gel 60 H supplied by Merck, and by argentation chromatography (Houx et al., 1974). All products were at least 98.5% pure and at least 99.7% pure with respect to geometric isomers as determined by capillary GLC on a Supelcowax 30-m or DB-Wax 30-m column.

Saturated acetates used for the methodological studies were from different sources, with the overall chemical purity always more than 95%.

Mass spectra were recorded on a Finnigan 4021 mass spectrometer, ^{1}H and ^{13}C NMR spectra on a Varian XL-300, a Nicolet 360 WB, or a Jeol FX-

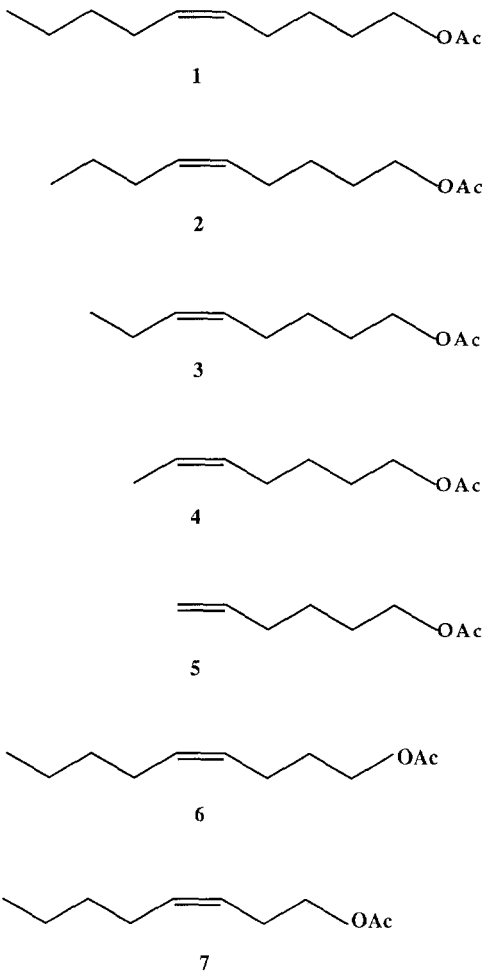


FIG. 1. Compounds studied.

60 spectrometer. NMR spectra were recorded on $CDCl_3$ solutions with Me_4Si as internal reference. For some of the products, the ^{13}C signal for the carbonyl carbon was of too low an intensity to be unambiguously identified.

DMPU was purchased from Fluka AB. Immediately before use, it was distilled over CaH_2 at reduced pressure and kept over 4 Å molecular sieves under an argon atmosphere.

(Z)-5-Decenyl Acetate (1). This was prepared as previously described (Olsson et al., 1983).

(Z)-5-Nonenyl Acetate (2). Ethyl 4-bromobutyrate was reduced with lith-

ium aluminium hydride, and the resulting bromoalcohol was protected with dihydropyran, according to standard procedures, affording 4-(2-tetrahydropyranyloxy)-1-bromobutane that was distilled at reduced pressure in base-washed glassware, bp 66–73°C/0.2 mm Hg. To a solution of liquid ammonia (100 ml) and dry ether (10 ml) at −78°C under a nitrogen atmosphere, lithium amide (0.92 g, 0.04 mol) was added in portions. The mixture was stirred for 20 min, and 1-pentyne (2.11 g, 0.03 mol) was added, according to a method described for similar systems by Berger and Canderday (1968). After stirring for an additional 45 min, 4-(2-tetrahydropyranyloxy)-1-bromobutane (5.00 g, 0.02 mol) dissolved in dry ether (25 ml) was slowly added. The resulting mixture was stirred for 2 hr at −78°C and then allowed to slowly warm up to room temperature while the ammonia evaporated. Water (50 ml) was added, and the resulting solution was extracted three times with hexane. The combined organic layers were washed with saturated NaCl solution and dried over $MgSO_4$. Removal of the solvent gave the crude product (4.04 g, 89%). Reduction with Lindlar catalyst (Lindlar, 1952; Maurer and Grieder, 1977; Leznoff et al., 1977; Marvell and Li, 1973; Wong et al., 1984), followed by acetylation (Schwartz and Waters, 1972) of the tetrahydropyranyl ether gave **2.** δ_H (300 MHz) 0.90 (t, 3H, Me), 1.31–1.46 (m, 4H, CH_2CH_2), 1.59–1.69 (m, 2H, $CH_2{-}C{-}O$), 1.96–2.10 (m, 4H, $CH_2C{=}$), 2.04 (s, 3H, Me−COO), 4.06 (t, 2H, $CH_2{-}OCO$), 5.34–5.40 (m, 2H, J_{AB} = 10.5 Hz, CH=CH). δ_C (75.4 MHz) 13.8, 20.9, 22.8, 26.0, 26.7, 28.2, 29.1, 64.5, 129.2, 130.3, 171.2. *m/z*: 124 (M^+-60; 7%), 109(1), 96(24), 81(35), 67(37), 61(2), 54(45), 43(100).

(Z)-5-Octenyl Acetate (3). 4-(2-Tetrahydropyranyloxy)-1-bromobutane (17.6 g, 0.074 mol) was reacted with lithium acetylide, ethylenediamine, complex, affording 14.4 g (62%) of 1-(2-tetrahydropyranyloxy)-5-hexyne (Smith and Beumel, 1974; Rossi et al., 1980; Jäger, 1977). Butyllithium (6 ml of a 1.44 M solution in hexane) was slowly added to 1-(2-tetrahydropyranyloxy)-5-hexyne (1.5 g, 8.2 mmol), dissolved in dry THF (8 ml). The solution was stirred at room temperature for 2 hr. Ethyl iodide (2.0 g, 0.013 mol) dissolved in DMPU (14 ml, freshly distilled from CaH_2) was added at such a rate that the temperature did not exceed 25°C (Bengtsson and Liljefors, 1988). The solution was stirred for 3 hr, then poured into ice water (50 ml) and extracted with hexane. The combined hexane layers were washed with saturated NaCl solution and dried over $MgSO_4$. After removal of the solvent, 1.69 g (92%) of crude 1-(2-tetrahydropyranyloxy)-5-octyne was obtained. After hydrogenation with Lindlar catalyst and acetylation, **3** was obtained. δ_H (300 MHz) 0.95 (t, 3H, Me), 1.38–1.46 (m, 2H, CH_2CH_2), 1.59–1.68 (m, 2H, $CH_2{-}C{-}O$), 2.01–2.10 (m, 4H, $CH_2C{=}$), 2.04 (s, 3H, Me−COO), 4.06 (t, 2H, $CH_2{-}OCO$), 5.28–5.41 (m, 2H, J_{AB} = 10.7 Hz, CH=CH). δ_C (75.4 MHz) 14.3, 20.5, 21.0, 26.0, 26.6, 28.1, 64.4, 128.4, 132.1, 171.2 *m/z*: 110 (M^+-60; 9%), 95(6), 82(40), 67(52), 61(2), 55(19), 43(100).

(Z)-5-Heptenyl Acetate (4) was prepared from 1-(2-tetrahydropyranyloxy)-5-hexyne and methyl iodide, according to the same procedure as described above for **3.** δ_H (300 MHz) 1.36–1.47 (m, 2H, CH_2CH_2), 1.58–1.69 (m, 5H, MeC=, CH_2—C—O), 2.03–2.11 (m, 2H, CH_2C=), 2.05 (s, 3H, Me—COO), 4.06 (t, 2H, CH_2—OCO), 5.32–5.52 (m, 2H, J_{AB} = 10.6 Hz, CH=CH). δ_C (75.4 MHz) 12.7, 21.0, 25.8, 26.3, 28.1, 64.5, 124.3, 130.0, 171.3. *m/z*: 96 (M^+-60; 11%), 81(20), 68(64), 61(3), 55(27), 43(100).

5-Hexenyl Acetate (5). 1-(2-Tetrahydropyranyloxy)-5-hexyne was reduced with disiamyl borane (Brown and Zweifel, 1961) and acetylated. δ_H (300 MHz) 1.37–1.48 (m, 2H, CH_2CH_2), 1.56–1.66 (m, 2H, CH_2—C—O), 2.00–2.09 (m, 2H, CH_2C=), 2.01 (s, 3H, Me—COO), 4.03 (t, 2H, CH_2—OCO), 4.91–5.02 (m, 2H, C=CH_2), 5.70–5.83 (m, 1H, CH=C). δ_C (75.4 MHz) 20.9, 25.1, 28.0, 33.2, 64.4, 114.8, 138.3, 171.2. *m/z*: 82 (M^+-60; 13%), 73(2), 67(29), 61(2), 54(35), 43(100). $C_8H_{14}O_2$ calc. C 67.6, H 9.9; found C 67.3, H 9.9.

(Z)-4-Nonenyl Acetate (6). 3-(2-Tetradropyranyloxy)-1-brompropane (prepared from 1-bromo-3-propanol and dihydropyran) was coupled with hexyne in liquid ammonia–ether as described for **2.** δ_H (300 MHz) 0.90 (t, 3H, Me), 1.28–1.35 (m, 4H, CH_2CH_2), 1.63–1.73 (m, 2H, CH_2—C—O), 1.99–2.15 (m, 4H, CH_2C=), 2.05 (s, 3H, Me—COO), 4.06 (t, 2H, CH_2—OCO), 5.31–5.43 (m, 2H, J_{AB} = 11.2 Hz, CH=CH). *m/z*: 124 (M^+-60; 9%), 109(1), 95(15), 81(38), 68(49), 61(1), 54(40), 43(100).

(Z)-3-Octenyl Acetate (7). 2-(2-Tetrahydropyranyloxy)-1-bromoethane (prepared from 1-bromoethanol and dihydropyran) was coupled with hexyne in liquid ammonia/ether as described for **2.** δ_H (300 MHz) 0.90 (t, 3H, Me), 1.30–1.36 (m, 4H, CH_2CH_2), 2.01–2.06 (m, 2H, CH_2C=), 2.04 (s, 3H, Me—CO), 2.36–2.41 (m, 2H, O—C—CH_2—C=), 4.06 (t, 2H, CH_2—OCO), 5.32–5.38 (m, 1H, J_{AB} = 11.2 Hz, CH=C). 5.46–5.52 (m, 1H, J_{AB} = 11.2 Hz, CH=C). *m/z*: 110 (M^+-60; 8%), 95(3), 81(17), 73(2), 68(20), 54(46), 43(100).

Electrophysiology. The biological activities of the pheromone component and of the analogs were investigated by the single sensillum technique (Kaissling, 1974), in which action potentials generated in olfactory sensilla on the male moth antenna were recorded. The method used was modified according to van der Pers and Den Otter (1978). Two days after emergence of the male moth, one of the antennae was excised and mounted in a grounded pipet electrode filled with Beadle-Ephrussi Ringer solution. To establish electrical contact with the olfactory neurons, the tip of a pheromone-sensitive sensillum was cut off. The Ringer-filled recording electrode was placed over the cut surface and connected to a high-impedance amplifier by an Ag–AgCl wire. The responses were stored on a Racal four-channel tape recorder and visualized on a storage oscilloscope.

The stimulus was applied to a piece of filter paper and put into a 5-ml plastic disposable syringe. Two milliliters of the syringe atmosphere was

injected by a hydraulic injection device (Murphy Developments, Hilversum, The Netherlands) into a purified and moistened air stream flushing over the preparation at a speed of 0.5 m/sec. For each stimulus concentration, 10 replicates were recorded.

Olfactory receptor cells specifically tuned to (*Z*)-5-decenyl acetate (**1**) are present in antennal sensilla type SW1 of *Agrotis segetum* and are readily accessible for single-cell recording (Hallberg, 1981; Löfstedt et al., 1982; van der Pers and Löfstedt, 1983). The receptor cell selective for (*Z*)-5-decenyl acetate could unambiguously be distinguished from the other receptor cells present in the sensillum by the amplitude of its action potential (Löfstedt et al., 1982). The responses of this receptor cell were recorded for all analogs.

The receptor cell response was defined by the number of action potentials generated during 1 sec from the onset of the stimulation. Dose–response curves were constructed using five different stimulus concentrations. For the pheromone component, **1**, the stimulus concentration ranged from 10^{-4} μg to 1 μg in decadic steps. For the analogs, the stimulus concentration ranged from 10^{-3} μg to 100 μg in decadic steps. The relative activity of each compound was expressed as the reciprocal of the relative quantities required to elicit the same response from the receptor cell, as the actual pheromone component elicited.

Stimulus Amounts Released from Odor Sources. The quantitative release of stimulus from the syringes used as odor sources (see above) was investigated by capillary gas chromatography on a Hewlett Packard 5830 GC equipped with a flame ionisation detector. Disposable syringes were loaded with stimulus applied to the filter paper in 100 μl of hexane. The syringe was fitted to an injection needle and 2 ml of the 5-ml syringe atmosphere was injected slowly (during approximately 1 min because of the limited flow capacity of the capillary column) splitless into the gas chromatograph. The initial temperature (60°C) was maintained for 2 min following the beginning of the injection. Then the split valve was opened, and the oven temperature was programmed at 5°C/min to 200°C. A 30-m × 0.25-mm-ID fused silica DB-1 column (J&W Scientific, Folsom, California 95630) was used for the separations. Hydrogen was supplied as carrier gas at 40 cm/sec linear velocity.

Six syringes containing a constant amount of the internal standard (*Z*)-5-decenyl acetate (500 μg) and different amounts of decyl acetate (30, 50, 100, 300, 500, and 1000 μg) were prepared to investigate the release of stimulus from odor sources loaded with different amounts of stimulus compound. The amount of decyl acetate relative to the internal standard was estimated in five consecutive injections for each dose. Absolute amounts released were estimated by comparison of peak areas with those obtained when known amounts of decyl acetate were injected split–splitless in 1–3 μl of hexane. In this experiment, the syringe atmosphere was allowed to equilibrate during the time of the preceding analysis (1 hr or more).

A mixture of six saturated acetates (hexyl, heptyl, octyl, nonyl, decyl, and undecyl) and the internal standard **1** was prepared to investigate the relation between volatility and relative amounts released from the odor sources. One hundred micrograms of each saturated acetate and 500 μg of the internal standard in 100 μl of hexane were applied to the odor source filter paper. In one experiment, the relative amounts released in five consecutive injections from the same syringe were investigated as described above. In a second experiment, the amounts released from five different, freshly prepared syringes were quantified. In this experiment, the hexane was allowed to evaporate, the syringe atmosphere were replaced once, and was then allowed to equilibrate during 2 min, before injection of the 2 ml on the GC.

RESULTS AND DISCUSSION

Dose–response curves in insect electrophysiology are usually based on amounts of stimuls applied to the odor source rather than the amount of stimulus to which the insect preparation is actually exposed. Quantification of the stimulus amounts released from the syringes used as odor sources in this and in our previous studies corroborated our assumption that the amounts released are proportional to the amounts applied to the filter paper (Figure 2) within the concentration range usually used in our experiments.

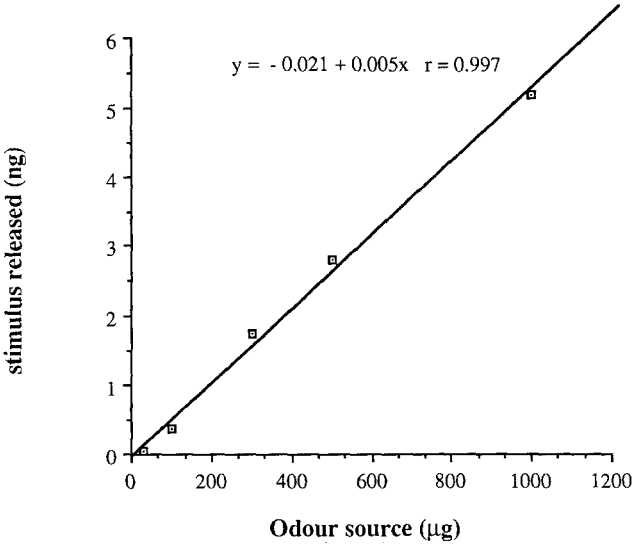


FIG. 2. Amounts of stimulus released from odor sources loaded with different amounts of decyl acetate on filter paper (N = 5).

The amount of a saturated acetate in 2 ml of syringe atmosphere was found to be proportional to the chain length of the acetate (Figure 3). The results of the two different experimental procedures shown in Figure 3A and B are essentially identical. The addition of one methylene unit on the average reduced the released amount by a factor of 3.9 [the average of exp (1.389) and exp (1.349)]. This factor is similar to the factor by which the saturated vapor pressures of straight-chain alkyl derivatives are reduced on the addition of a methylene group on the alkyl chain. Accurate vapor pressures for the entire series of alkyl acetates studied here are not available, but for lower homologs up to pentyl acetate,

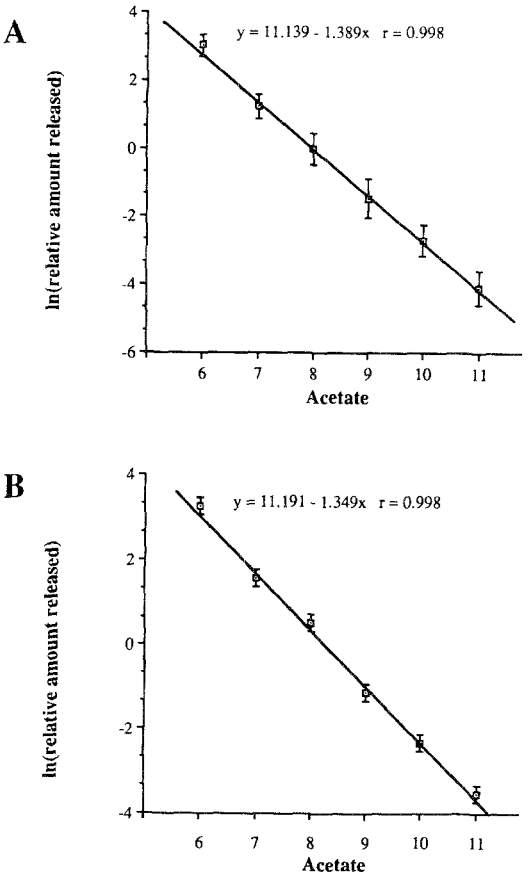


FIG. 3. Natural logarithm of relative amounts released of a homologous series of straight chain acetates (hexyl to undecyl acetate). The data points represent the average of injections from five consecutive injections from the same syringe (A) and five different freshly prepared syringes (B). (See text for further explanation.)

this factor is 2.8 (Jordan, 1954). The relative vapor pressures of decyl acetate (Olsson et al., 1983) and pentyl acetate correspond to a factor of 2.9 for each methylene group added to pentyl acetate. Thus, the relative amounts released from the odor source correspond to a reasonably good approximation to the relative saturated vapor pressures. The relative vapor pressures on chain shortening–chain elongation are similar for different classes of straight-chain alkyl derivatives. For a homologous series of alkanes (6–16 carbons), the corresponding factor is 3.2 (Stein, 1981). For straight-chain aldehydes (8–12 carbons) it is 3.7, and for 1-alkenes (10–13 carbons) it is 3.4 (Dykyj and Rep'as, 1979). Olsson et al. (1983) reported an average factor of 2.6 in the relative vapor pressures of monoenic acetates differing by one methylene unit. This indicates that relative standard vapor pressures may be used to estimate the relative amounts of compounds, differing in volatility, actually released from an odor source.

Our results demonstrate the necessity of corrections for differences in volatility when the electrophysiological activity of compounds with different chain lengths and/or vapor pressures are compared. For instance, with equal amounts of hexyl and decyl acetate at the odor source, approximately 231 times more hexyl acetate will stimulate the antenna if a chain length constant of 3.9 is used. Regardless of the exact value of the constant, corrected stimulus amounts are much more accurate than uncorrected ones.

The electrophysiological single-cell activities for compounds **1–7,** including corrections for volatility differences, are shown in Figure 4. All the chain-shortened analogs show a decrease of the biological activity compared to the natural pheromone component **1.** For the analogs shortened in the n-chain (Scheme 2), a loss of activity is found for each additional CH_2 group removed (compounds **2–5**). The loss of activity is about a factor of 16 for the removal of the first methylene group (compound **2**), and a factor of 6 for the removal of the second one (compound **3**). With further shortening of the *n* chain, the electrophysiological activity decreases drastically, finally giving the essentially inactive compound **5.**

These results are in line with those obtained by Priesner (1979a,b) in EAG screenings of several species of Noctuidae and Tortricidae. For five species, analogs chain shortened by one methylene unit showed activities 5.6–18 times less than those for the natural pheromone component. The analogs chain shortened by two and three methylene units were found to be 18–180 and 180–560 times less active, respectively. On stimulation of a male receptor cell of the

SCHEME 2.

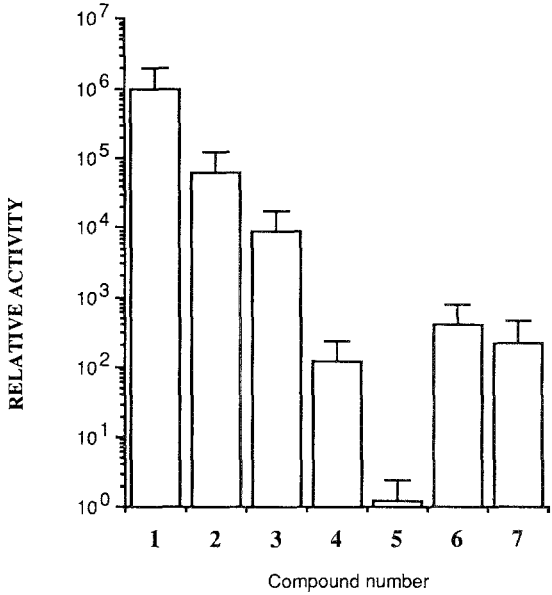


FIG. 4. Experimental single-cell activities for compounds **1–7.** The data are corrected for differences in vapor pressure. Error bars show maximal errors.

tortricid moth, *Adoxophyes orana*, selective for (Z)-9-tetradecenyl acetate, with (Z)-9-dodecenyl acetate, Priesner (1983) observed an activity loss of a factor of 30. Similarly, (Z)-7-decenyl acetate is about 25 times less active than the natural substrate on the (Z)-7-dodecenyl acetate receptor of *Agrotis segetum* (Liljefors et al., 1984). It should be noted that these data from the literature do not take differences in volatility into account.

The results shown in Figure 4 indicate that although the chain length of the natural component **1** is the optimal one, compounds one and two methylene units shorter can interact with and activate the receptor to a significant degree. For instance, compound **3** which is chain shortened by two methylene units, is 10 times as active as the corresponding compound chain elongated by two methylene units (Liljefors et al., 1987). The quite high activities of compounds **2** and **3** indicate that not only the terminal methyl group but also at least the next two methylene groups in the chain may efficiently interact with a hydrophobic region of the assumed receptor cavity, a region which is complementary to the van der Waals surface of the substrate molecule. Thus, the shape of the part of the receptor cavity interacting with the *n* chain may be a deep "groove" or "pocket," at least partly circumscribing the aliphatic chain. Figure 5 shows a clipped dot-surface representation of the van der Waals surface of part of the *n*

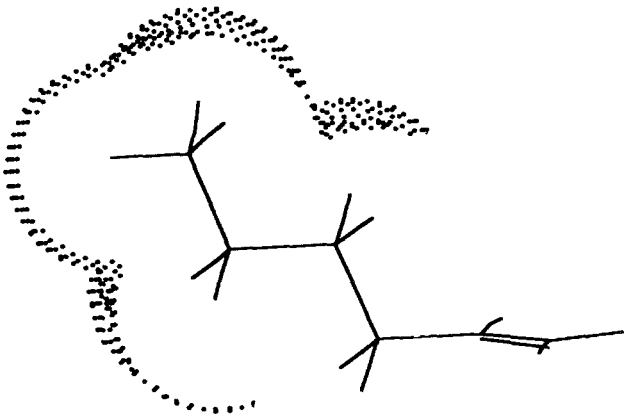


FIG. 5. A cross-section of the van der Waals surface of part of the terminal alkyl chain in compound **1**, indicating the size and shape of the complementary receptor cavity area interacting with this part of the molecule.

chain indicating a possible shape of this region of the receptor cavity. The lower part of the surface in Figure 5 is left undefined. It should correspond to a cavity large enough to accommodate the chain loops formed by chain-elongated analogs according to our receptor–interaction model (Liljefors et al., 1987).

From binding experiments on various enzymes, a maximum binding energy of 3.2 kcal/mol has been evaluated for a methyl group interacting with a site with "perfect" complementarity (Fersht, 1985). This value for hydrophobic binding of a methyl group is somewhat larger than that obtained from free energies of transfer of hydrocarbons from aqueous solution to pure liquid hydrocarbon, 2.1 kcal/mol (Tanford, 1980). Since the cavity in the enzyme is preformed, while the cavity in solution must be created to accommodate the solute, this is to be expected.

In order to make comparisons with binding energies possible, conformational entropies must be taken into account. The chain-shortened analogs **2–5** have one single bond less for each methylene group removed compared to the parent compound **1.** If we assume, as in previous studies (Liljefors et al., 1985, 1987; Bengtsson et al., 1987), that the molecule is rigidly bound to the receptor, the loss of conformational entropy on binding to the receptor is smaller for a chain-shortened analog of **1** than for **1.** Assuming an entropy loss of ca. 4 cal/mol K for "freezing" an internal rotation (Winnik, 1981; Liljefors et al., 1985), this difference may be estimated to be ca. 1 kcal/mol for each methylene unit removed. The maximum binding energy ("perfect" complementarity) for the terminal methyl of compound **1** then becomes $3.2 - 1.0 = 2.2$ kcal/mol.

Previously we found that an increase of the interaction energy of 1.6 kcal/

mol corresponds to an activity decrease by a factor of 10 (Liljefors et al., 1987; Bengtsson et al., 1987). As the activity drops by a factor of 16 on going from compound **1** to compound **2** (Figure 4), this corresponds, according to our model, to a loss of 1.9 kcal/mol of interaction energy. Note that relative activities reflect differences in total interaction energies. The total interaction energy is a sum of intermolecular interaction energies due to hydrophobic interaction, hydrogen bonding, and so on, subtracted by the conformational energy needed for the substrate molecule to acquire the "correct" conformation. In our previous work on compounds that have the ability to mimic the natural pheromone component, differences in total interaction energies only depend on the conformational energy term (Liljefors et al., 1987; Bengtsson et al., 1987). However, for chain-shortened analogs, differences in total interaction energies also depend on the intermolecular interaction energy term.

Shortening of compound **1** by one methylene group removes the methyl group from its interaction site (see Figure 5). Since the calculated energy loss is close to the value for maximum binding energy of a methyl group as derived above, this strongly indicates that the terminal methyl group is interacting with a very high degree of complementarity with its receptor site in the receptor cavity. This supports our model, in which the terminal methyl group of the natural pheromone component **1** is assumed to interact with a very well-defined receptor site (Liljefors et al., 1987).

In our receptor–interaction model, the double bond is also assumed to interact with a well-defined receptor site. This implies that the terminal methyl group in compound **2** cannot interact with the methyl group site in the receptor cavity—the *n* chain is too short. Providing that the "pocket" that binds the *n* chain extends to the methylene unit in the 8-position, shortening of the *n* chain in **2** by one methylene group to give compound **3** should result in a somewhat smaller activity loss than for the corresponding shortening of compound **1.** The decrease in activity should correspond to the loss of hydrophobic interactions of one methylene group, which in energy terms is about one third of the hydrophobic binding energy for a methyl group (Tanford, 1980). The experimental data support this conclusion: the activity drops by only a factor of 6 on going from compound **2** to compound **3** (Figure 4). On further shortening of the *n* chain (compounds **4** and **5**), the observed low activities (Figure 4) indicate that the alkyl groups have no interactions, or only very weak interactions, with the receptor.

When chain-shortening is done in the *m* chain (compounds **6** and **7,** Figure 4), the loss of electrophysiological activity is more dramatic. The removal of one or two CH_2 groups from the *m* chain results in a decrease of the biological activity by a factor of about 3000. From extensive EAG investigations of noctuid moths, Bestmann and Vostrowsky (1982) concluded that structural variations of the alkyl parts of a pheromone component of the investigated species

produce a more drastic loss of activity if the *n* chain is varied than when the equivalent changes are made in the *m* chain. This "structure–activity rule" is not in agreement with the results in Figure 4, and we have previously found that this "rule" is also not in agreement with experimental data for dienic analogs of (Z)-5-decenyl acetate (Bengtsson et al., 1987).

Interestingly, the experimental single-cell data (Figure 4) show that essentially the same loss of activity is obtained with the removal of one or two CH_2 units from the *m* chain. This was also observed by Priesner (1979a,b) in the EAG screening relative to above. Similar observations were done in a single-cell study on pheromone components of some saturniid species (Bestmann et al., 1987). In the single-cell study of *Adoxyphyes orana* mentioned above, Priesner (1983) found that a shortening of the *m* chain of (Z)-9- and (Z)-11-tetradecenyl acetate by two methylene units resulted in an activity decrease by factors of 100 and 30, respectively. When these values are corrected for differences in volatility, the first one is similar to the results we obtain for the corresponding compound **7.**

In our study on modifications of the acetate group and the corresponding effects on the electrophysiological activity (Liljefors et al., 1984), we found very strict requirements on the shape as well as on the electron distribution of the polar functional group for a productive interaction with the receptor. The results in Figure 4 are consistent with this conclusion. If the receptor cavity is highly complementary to the acetate group in **1,** as illustrated in Figure 6, a shortening of the *m* chain in this compound with one methylene group essentially destroys all interactions between the acetate group and the receptor. As shown in Figure 7, the orientation of the acetate group is very different in the

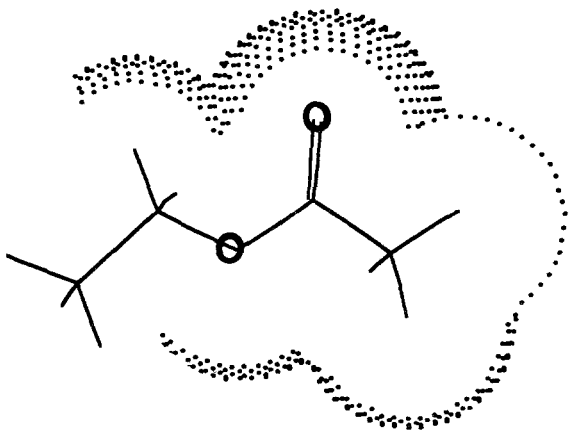


FIG. 6. A cross section of the van der Waals surface of part of the acetate group.

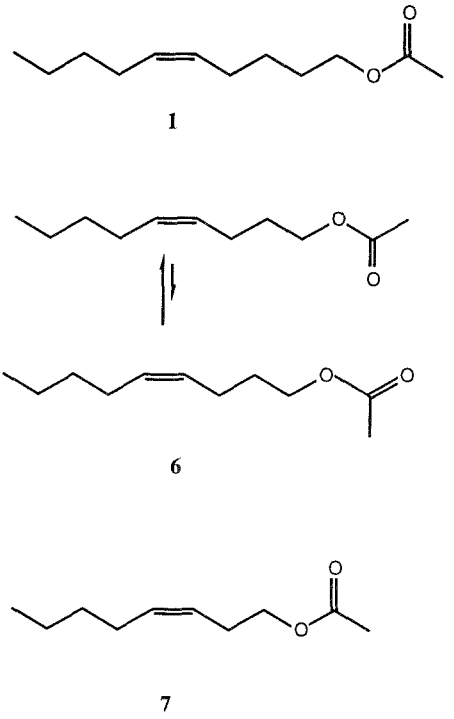


FIG. 7. Orientations of the acetate group in compounds **1**, **6**, and **7**.

thermodynamically preferred conformers of compounds **1** and **6**. We have found previously that the direction of the dipole moment of the polar functional group is of decisive importance for a productive receptor interaction (Liljefors et al., 1984). The direction of the dipole moment of the acetate group is approximately parallel to the carbonyl group. Thus, these dipole moment directions are antiparallel in **1** and **6**, which is incompatible with a productive receptor interaction for **6**. Rotation about the C—O bond in **6** (Figure 7) changes the dipole moment direction of its acetate group, but it is still far away from the optimal direction. Furthermore, such a conformational rearrangement is of prohibitively high energy, 8.5 ± 1.0 kcal/mol (Blom and Günthard, 1981).

Since the type of interaction between the acetate group in **1** and the receptor is not known, it is not possible to calculate the loss of interaction energy on going from compound **1** to **6**. However, as all parts of the acetate group seem to contribute to this interaction energy (Liljefors et al., 1984), this loss should be greater than the loss of interaction energy on removal of a methyl group from its site as discussed above.

In compound **7** the direction of the dipole moment of the acetate group is

the same as in **1** (Figure 7). However, an optimal interaction with the receptor sites by this group removes the double bond and the terminal methyl group from their sites. It is more likely that these interactions are as in **1** and that the acetate group only very weakly interacts with the receptor sites occupied by the acetate group in compound **1.** The experimental data in Figure 4 support this conclusion. Shortening the *m* chain in **6** to give compound **7** should, in this case, only reduce the activity by a factor corresponding to, at most, the loss of the hydrophobic interactions of one methylene group. As discussed above, for the *n* chain this corresponds to an activity drop by about a factor of 6. The observed activity for **7** is lower by a factor of about two compared to that for **6** (Figure 4), which is in reasonably good agreement with this prediction.

CONCLUSIONS

The terminal methyl group as well as the acetate group in the natural pheromone component **1** are concluded to interact with highly complementary sites in the receptor cavity. Thus, the dimensions of the receptor cavity in this respect correspond very closely to the length of the natural substrate. Our analysis of the experimental single-cell data indicates that the *n* chain binds to a hydrophobic "pocket" that extends over the two methylene groups closest to the terminal methyl group. Thus, compounds that are chain shortened by one or two methylene units have sufficiently strong interactions with the hydrophobic "pocket" to activate the receptor to a significant degree.

Chain shortening of one methylene unit in the *m* chain leads to a large drop in activity, probably due to the more or less complete removal of the strong interaction between the acetate group and its receptor sites. Further shortening of the *m* chain leads to a much lower activity loss due to loss of only hydrophobic interactions.

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