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STRUCTURE–ACTIVITY CORRELATIONS AMONG ANALOGS OF THE CURRANT CLEARWING MOTH PHEROMONE

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Abstract—Eleven analogs of (*E*,*Z*)-2,13-octadecadien-1-yl acetate **1**, a main pheromone component of the currant clearwing moth, *Synanthedon tipuliformis* Clerk (Lepidoptera: Sesiidae) were synthesized and tested for their biological activities by electroantennography (EAG). To correct the EAG data for differences in volatility of the analogs, their vapor pressures were estimated by a gas chromatographic method. All structural changes in the parent molecule were found to reduce the biological activity to various degrees. The most active analog tested was the carbamate **12**, whose activity was almost comparable to that of the pheromone component **1**. Structure–activity correlations showed that hydrophobic, steric, and electronic effects of chain terminal groups might be responsible for variations in biological activity of the conformationally unchanged (*E*,*Z*)-2,13-analogs

Key Words—Pheromone analogs, (*E*,*Z*)-2,13-octadecadien-1-yl acetate, EAG, gas chromatography, vapor pressure, currant clearwing moth, *Synanthedon tipuliformis* Clerk, Lepidoptera, Sesiidae.

INTRODUCTION

Pheromone analogs are known to have potential for investigation of the olfactory transduction mechanism and for use in alternative pest control strategies (Prestwich, 1987; Evershed, 1988). It is now generally accepted that the receptor cavity contains highly complementary interaction sites to the three key molecular

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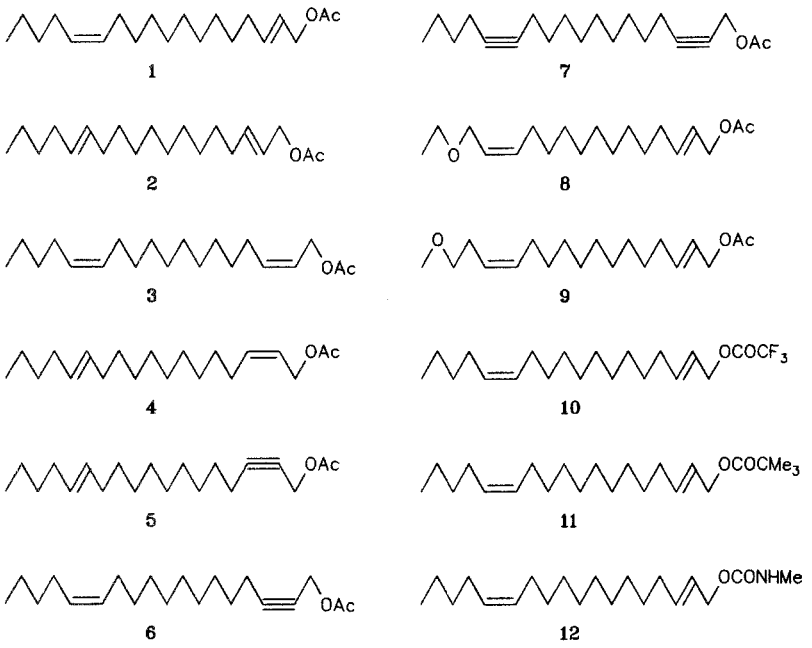
parts of the stimulus molecule, i.e., to the double bond, the terminal alkyl group, and the polar functional group. However, most of the structure–activity studies have been focused on relatively short monounsaturated acetates, e.g., (*Z*)-5-decen-1-yl and (*Z*)-7-dodecen-1-yl acetates (Liljefors et al., 1984, 1985, 1987; Bengtsson et al., 1987, 1990; Jönsson et al., 1991a,b), sex pheromone components of the turnip moth, *Agrotis segetum.* Specialized studies on longer-chain, unconjugated dienic acetates are rather rare and not nearly exhaustive enough (Bestmann et al., 1987) to allow generalizations.

In this study, the currant clearwing moth (CCM), *Synanthedon tipuliformis* Clerk (Lepidoptera: Sesiidae), which is a serious pest of red and black currant and gooseberry in Europe as well as in Asia, North America, and Australia, was selected as a target species. Its female sex pheromone has been identified (Arn et al., 1986) as a two-component mixture consisting of (*E*,*Z*)-2,13-octadecadien-1-yl acetate (**1**, Scheme 1) (94%) and the corresponding C_2-saturated analog, (*Z*)-13-octadecen-1-yl acetate. Several efficient syntheses of **1** have recently been reported (Hoskovec et al., 1990; Sharma et al., 1990; Sorochinskaya and Kovalev, 1991) and its attractant activity confirmed in field tests (Szöcs et al., 1990). Recently, the intraspecific variability in CCM pheromone communication has been observed (Szöcs et al., 1991). Depending on the geo-



SCHEME 1.

graphic region, trace amounts of the (*E*,*Z*)-3,13-isomer were found to cause either synergistic or inhibitory effects.

In an effort to provide clues to the spatial and/or functional group requirements for biological activity of the pheromone component **1,** we conducted an electrophysiological study on the response of CCM males to 11 analogs of **1** (Scheme 1). To corroborate the results of EAG dose–response measurements with respect to differences in volatility between test compounds, vapor pressures of all analogs were estimated by a simple gas chromatographic method and used to correct the original EAG data.

The analogs **2–12** (Scheme 1) proceed from modifications in the key molecular parts that may be directly involved in the interaction process with the receptor. Thus, all the possible double bond configuration changes at positions **2** and **13** are reflected in compounds **2–4.** In the acetylene derivatives **5–7,** one or two double bonds are replaced by a triple bond, whereas in oxa analogs **8** and **9** one of the parafinic carbons is substituted by an oxygen atom. Finally, in esters **10–12,** the acetate moiety is replaced by the trifluoroacetate, pivalate, and *N*-methyl carbamate groups, respectively.

METHODS AND MATERIALS

Insects

Diapausing larvae hidden in currant stems were collected from red and black currant plantages in South Bohemia in March 1991. The larvae were kept outdoors until emergence. Moths hatched in June were collected daily and sexed. The males were held separately in Petri dishes and provided water. Three day-old individuals were used for EAG experiments.

Electrophysiological Recording

Recordings were made from antennae of intact, mechanically immobilized moths. Ag–AgCl electrodes filled with Ringer solution were used to record slow negative EAG potentials generated by olfactory stimulation. The recording electrode was positioned at a cut distal end of the antenna. The EAG potentials were amplified by a high-impedance DC amplifier, monitored on a dual-beam storage oscilloscope, and recorded by a pen recorder. The maximal negative EAG deflections were evaluated.

Stimulation

Serial dilutions of the pheromone component and its analogs were prepared in hexane. From each solution, 5 μl was soaked in a filter-paper disk of 10 mm diameter. After solvent evaporation, the loaded disks were individually inserted

into Pasteur pipets and stored in closed glass vials at −20°C. One hour prior to the experiments, the stimulus cartridges were transferred to attain room temperature. The antennal preparation was continuously blown over by charcoal-filtered and humidified air (1 liter/min). During 0.5-sec stimulation, a constant volume (≈8.3 ml) of air was injected through the stimulus cartridge upon the antenna. The stimuli were delivered from the lowest to the highest concentrations, with 2-min pauses between stimuli at lower concentrations and 5–15 min at concentrations greater than 10^{-1} g/liter. These pauses were adequate for complete recovery of the EAG and for readaptation of receptors after previous olfactory stimulation.

EAG Evaluation

Doses of 10^{-1} g/liter of (*E*,*Z*)-2,13-octadecadien-1-yl acetate **1** were used as standard to (1) normalize responses, and (2) control for viability and constancy of the preparation. Stimulation with the standard both preceded and followed each serial dilution level. Each EAG response to test chemicals was expressed as a percentage of the mean of the two nearest responses to the standard.

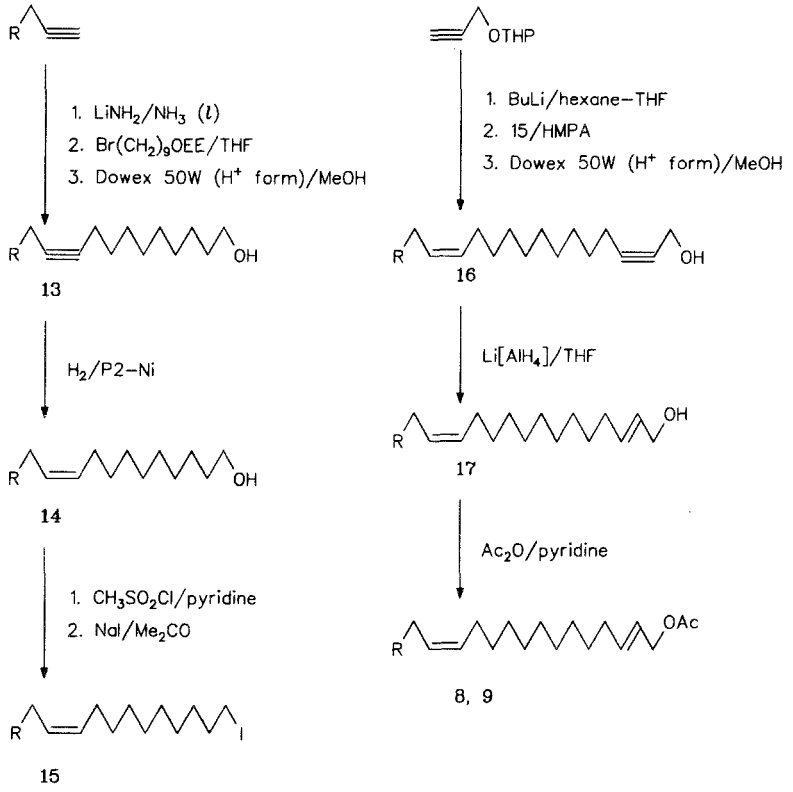
Chemicals

Acetates **1–7** were prepared by previously reported methods (Hoskovec et al., 1990). Trifluoroacetate **10,** pivalate **11,** and *N*-methyl carbamate **12** have been prepared by standard esterifications of the parent (*E*,*Z*)-2,13-octadecadien-1-ol. Their IR, NMR, and mass spectra were fully consistent with the proposed structures. All the doubly unsaturated final alcohols were ultimately purified by argentation chromatography (Voerman and Rothschild, 1978; Voerman et al., 1984) and checked by GC. Their purity was estimated to be 99%. The synthesis of the oxa analogs **8** and **9** is described below (Scheme 2).

^{1}H NMR spectra were determined in $CDCl_3$ solution on Varian UNITY-500 and UNITY-200 spectrometers, operating at 499.5 and 200.1 MHz, respectively, and absorptions are expressed on a δ (ppm) scale relative to TMS. GLC analyses were performed on a Hewlett Packard HP 5880A chromatograph equipped with a FID detector and a 25-m capillary column (internal diameter 0.3 mm, HP5-5% phenyl methylsilicone, cross-linked). Flash chromatography separations were made on Merck 60 silica gel (0.040–0.063 mm) using a Büchi B-680 Prep LC System with a stepwise gradient of ethyl acetate in light petroleum.

13-Oxa-10-pentadecyn-1-ol ***(13a)***

To a suspension of lithium amide (prepared from 1.47 g of lithium and 1000 ml of liquid ammonia) 23.0 g (0.273 mol) of 4-oxa-1-hexyne (Camps et al., 1988) in dry tetrahydrofuran (300 ml) was introduced. After stirring for 1.5



In formulae 8, 13–17a: R= CH_3CH_2O–
9, 13–17b: R= CH_3OCH_2–

SCHEME 2.

hr 1-(1-ethoxyethyloxy)-9-bromononane (51.7 g, 0.175 mol) in dry tetrahydrofuran (300 ml) was added dropwise and stirring was continued for 4 hr. Ammonia was evaporated on standing overnight, and the residue was decomposed with ice-cold water. The mixture was then extracted with ether (4 × 300 ml), and the ethereal extracts were washed with brine and dried over potassium carbonate. Evaporation of the solvent furnished 41.6 g of a red oil, which was dissolved in methanol (1500 ml) and treated with Dowex W50 (H^+ form; 30 g) for 24 hr. The ion exchanger was filtered off and the solvent removed in vacuo. Flash chromatography of the residue yielded 25.9 g (65%) of alkynol **13a.**

Analysis. Calcd. for $C_{14}H_{26}O_2$: C, 74.29; H, 11.58. Found.: C; 74.03, H, 11.41. ^1H NMR: δ 1.23 (t, J = 7.1 Hz, 3H, $C\underline{H}_3CH_2O$—), 1.23–1.43 (m,

12H, $-CH_2-$), 1.53–1.56 (m, 2H, $-C\underline{H}_2CH_2OH$), 2.21 (tt, J = 7.0, 2.2 Hz, 2H, $-C{\equiv}CC\underline{H}_2-$), 3.55 (q, J = 7.1 Hz, 2H, $CH_3C\underline{H}_2O-$), 3.64 (t, J = 6.6 Hz, 2H, $-C\underline{H}_2OH$), 4.12 (t, J = 2.2 Hz, 2H, $-OC\underline{H}_2C{\equiv}C-$).

14-Oxa-10-pentadecyn-1-ol ***(13b)***

Analysis. Calcd. for $C_{14}H_{26}O_2$: C, 74.29; H, 11.58. Found.: C; 74.41, H, 11.73. ^{1}H NMR: δ 1.26–1.68 (m, 14H, $-CH_2-$), 2.18 (tt, J = 2.4, 7.1 Hz, 2H, $-C{\equiv}CC\underline{H}_2-$), 2.47 (tt, J = 2.4, 7.1 Hz, 2H, $-OCH_2C\underline{H}_2C{\equiv}C-$), 3.41 (s, 3H, $C\underline{H}_3O-$), 3.51 (t, J = 7.1 Hz, 2H, $-OC\underline{H}_2CH_2C{\equiv}C-$), 3.68 (t, J = 6.3 Hz, 2H, $-C\underline{H}_2OH$).

(Z)-13-Oxa-10-pentadecen-1-ol ***(14a)***

1,2-Diaminoethane (3.2 g) and **13a** (25.0 g; 0.110 mol) of were added to a suspension of P2-Ni [prepared from 1.98 g of nickel(II) acetate] in ethanol (250 ml) and hydrogenated with stirring at 25°C (Brown and Ahuja, 1973). The hydrogenation was monitored by analyzing aliquots of the solution by GLC. Flash chromatography of the crude product afforded 24.3 g (97%) of (Z)-alkenol **14a.**

Analysis. Calcd. for $C_{14}H_{28}O_2$: C, 73.63; H, 12.36. Found.: C; 73.85, H, 12.29. ^{1}H NMR: δ 1.21 (t, J = 7.1 Hz, 3H, $C\underline{H}_3CH_2O-$), 1.24–1.40 (m, 12H, CH_2), 1.55–1.59 (m, 2H, $-C\underline{H}_2CH_2OH$), 2.06 (bq, J = 6.5 Hz, 2H, $-CH{=}CHC\underline{H}_2-$), 3.49 (q, J = 7.1 Hz, 2H, $CH_3C\underline{H}_2O-$), 3.64 (t, J = 6.6 Hz, 2H, $-C\underline{H}_2OH$), 4.00–4.04 (m, 2H, $-OC\underline{H}_2CH{=}CH-$), 5.51–5.59 (m, 2H, $-C\underline{H}{=}C\underline{H}-$).

(Z)-14-Oxa-10-pentadecen-1-ol ***(14b)***

Analysis. Calcd. for $C_{14}H_{28}O_2$: C, 73.63; H, 12.36. Found.: C; 73.49, H, 12.25. ^{1}H NMR: δ 1.27–1.65 (m, 14H, $-CH_2-$), 2.08 (bq, J = 6.6 Hz, 2H, $-CH{=}CHC\underline{H}_2-$), 2.37 (bq, J = 7.1 Hz, 2H, $-OCH_2C\underline{H}_2CH{=}CH-$), 3.39 (s, 3H, $C\underline{H}_3O-$), 3.42 (t, J = 7.1 Hz, 2H, $-OC\underline{H}_2CH_2CH{=}CH-$), 3.68 (t, J = 6.4 Hz, 2H, $-C\underline{H}_2OH$), 5.40 (dtt, J = 1.3, 6.9, 10.8 Hz, 1H, $-O(CH_2)_2CH{=}C\underline{H}-$), 5.52 (dtt, J = 1.3, 6.9, 10.8 Hz, 1H, $-O(CH_2)_2C\underline{H}{=}CH-$).

(Z)-1-Iodo-13-oxa-10-pentadecene ***(15a)***

This was obtained (Ramiandrasoa and Descoins, 1989) from **14a** mesylate (12.5 g; 0.041 mol) and NaI (12.2 g; 0.082 mol) in dry acetone (100 ml) with 85% (12.6 g) yield.

Analysis. Calcd. for $C_{14}H_{27}IO$: C, 49.71; H, 8.05; I, 37.51. Found.: C, 49.60; H, 8.12; I, 37.63. ^{1}H NMR: δ 1.21 (t, J = 7.1 Hz, 3H, $C\underline{H}_3CH_2O-$),

1.22–1.40 (m, 12H, $-CH_2-$), 1.77–1.83 (m, 2H, $-C\underline{H}_2CH_2I$), 2.04–2.08 (m, J = 6.6 Hz, 2H, $-CH=CHC\underline{H}_2-$), 3.19 (t, J = 6.9 Hz, 2H, $-C\underline{H}_2I$), 3.49 (q, J = 7.1 Hz, 2H, $CH_3C\underline{H}_2O-$), 4.00–4.04 (m, 2H, $-OC\underline{H}_2CH=CH-$), 5.49–5.61 (m, 2H, $-C\underline{H}=C\underline{H}-$).

(Z)-1-Iodo-14-oxa-10-pentadecene ***(15b)***

Analysis. Calcd. for $C_{14}H_{27}IO$: C, 49.71; H, 8.05; I, 37.51. Found.: C, 49.82; H, 8.11; I, 37.39. ^{1}H NMR: δ 1.23–1.42 (m, 12H, $-CH_2-$), 1.79–1.83 (m, 2H, $-C\underline{H}_2CH_2I$), 2.04 (bq, J = 6.4 Hz, 2H, $-CH=CHC\underline{H}_2-$), 2.33 (bq, J = 7.0 Hz, 2H, $-OCH_2C\underline{H}_2CH=CH-$), 3.19 (t, 2H, J = 7.1 Hz, $CH_3OC\underline{H}_2-$), 3.35 (s, 3H, $C\underline{H}_3O-$), 3.38 (t, J = 6.9 Hz, 2H, $-C\underline{H}_2I$), 5.35 [dtt, J = 1.5, 6.9, 10.7 Hz, 1H, $-O(CH_2)_2CH=C\underline{H}-$], 5.50 (dtt, J = 1.5, 6.9, 10.7 Hz, 1H, $-O(CH_2)_2C\underline{H}=CH-$).

(Z)-16-Oxa-13-octadecen-2-yn-1-ol ***(16a)***

Butyllithium (2.5 M solution in hexane; 12.0 ml; 30 mmol) was added to a stirred solution of 1-(2-tetrahydropyranyloxy)-2-propyne (4.21 g, 37.5 mmol) in tetrahydrofuran (100 ml) and HMPA (12 ml) at −20°C under argon (Ramiandrasoa and Descoins, 1989). After 1 hr, iodoalkene **15a** (5.0 g, 14.8 mmol) in HMPA (12 ml) was added dropwise, and the mixture was stirred for an additional 4 hr at room temperature. The solution was then poured into ice-cold water and extracted with light petroleum. After evaporation, the residue was treated as described in the synthesis of **13a.** Yield: 2.79 g (70%) of enynol **16a.**

Analysis. Calcd. for $C_{17}H_{30}O_2$: C, 76.64; H, 11.35. Found.: C, 76.72; H, 11.48. ^{1}H NMR: δ 1.21 (t, J = 7.0 Hz, 3H, $C\underline{H}_3CH_2O-$), 1.24–1.38 (m, 14H, $-CH_2-$), 2.09 (bq, J = 6.6 Hz, 2H, $-OCH_2CH=CHC\underline{H}_2-$), 2.21 (tt, 2H, J = 2.2, 7.0 Hz, $-C\underline{H}_2C\equiv C-$), 3.49 (q, J = 7.0, 2H, $CH_3C\underline{H}_2O-$), 3.98–4.04 (m, 2H, $-OC\underline{H}_2CH=CH-$), 4.25 (t, J = 2.2, 2H, $-C\underline{H}_2OH$), 5.52–5.60 (m, 2H, $-C\underline{H}=C\underline{H}-$).

(Z)-17-Oxa-13-octadecen-2-yn-1-ol ***(16b)***

Analysis. Calcd. for $C_{17}H_{30}O_2$: C, 76.64; H, 11.35. Found.: C, 76.50; H, 11.28. ^{1}H NMR: δ 1.23–1.85 (m, 14H, $-CH_2-$), 2.04 [bq, 2H, $-O(CH_2)_2CH=CHC\underline{H}_2-$], 2.21 (tt, 2H J = 2.2, 7.1 Hz, $-C\underline{H}_2C\equiv CCH_2OH$), 2.33 (bq, J = 6.9 Hz, 2H, $-OCH_2C\underline{H}_2CH=CH-$), 3.35 (s, 3H, $C\underline{H}_3O-$), 3.38 (t, J = 6.9 Hz, 2H, $CH_3OC\underline{H}_2-$), 4.19 (dt, J = 2.2, 15.1, 1H, $-C\equiv CC\underline{H}_2OH$), 4.30 (dt, J = 2.2, 15.1 Hz, 1H, $-C\equiv CC\underline{H}_2OH$), 5.36 [dtt, J = 1.5, 7.1, 10.6 Hz, 1H, $-O(CH_2)_2CH=C\underline{H}-$], 5.47 [dtt, J = 1.5, 7.1, 10.6 Hz, 1H, $-O(CH_2)_2C\underline{H}=CH-$].

(E,Z)-16-Oxa-2,13-octadecadien-1-yl acetate ***(8)***

Lithium alanate (1.21 g, 32 mmol) was added at −20°C under argon to a solution of 1.7 g (6.4 mmol) of alcohol **16a** in dry tetrahydrofuran (75 ml) (Attenburow et al., 1952). After heating for 10 hr at 60°C, the mixture was decomposed with cold water (50 ml) and 10% sulfuric acid (100 ml) and extracted with ether (3 × 100 ml). The ethereal extracts were washed with brine and dried ($MgSO_4$). Removal of the solvent in vacuo and purification of the residue by chromatography afforded 1.5 g (89%) of the (*E,Z*)-dienol **17a.** The isomeric purity of the product was better than 97%. Subsequent argentation chromatography (Voerman and Rothschild, 1978) and acetylation of **17a** gave **8** in 95% yield and 99% isomeric purity.

Analysis. Calcd. for $C_{19}H_{34}O_3$: C, 73.50; H, 11.04. Found.: C, 73.62; H, 11.11. ^{1}H NMR: δ 1.21 (t, J = 7.1 Hz, 3H, $C\underline{H}_3CH_2O-$), 1.23–1.41 (m, 14H, $-CH_2-$), 2.06 (s, 3H, $-OCOC\underline{H}_3$), 2.02–2.08 (m, 2H, $-OCH_2CH=CHC\underline{H}_2-$), 2.02–2.08 (m, 2H, $-C\underline{H}_2CH=CH_2OAc$), 3.49 (q, 2H, $CH_3C\underline{H}_2O-$), 3.99–4.05 (m, 2H, $-OC\underline{H}_2CH=CH-$), 4.51 (dq, J = 1.1, 5.4 Hz, 2H, $-C\underline{H}_2OAc$), 5.51–5.59 (m, 2H, $-OCH_2C\underline{H}=C\underline{H}-$), 5.55 (dtt, J = 1.0, 5.5, 15.4 Hz, 1H, $-CH=C\underline{H}-CH_2OAc$), 5.78 (dtt, J = 1.0, 6.5, 15.4 Hz, 1H, $-C\underline{H}=CH-CH_2OAc$).

(E,Z)-17-Oxa-2,13-octadecadien-1-yl acetate ***(9)***

Analysis. Calcd. for $C_{19}H_{34}O_3$: C, 73.50; H, 11.04. Found.: C, 73.31; H, 10.93. ^{1}H NMR: δ 1.23–1.41 (m, 14H, $-CH_2-$), 2.06 (s, 3H, $-OCOC\underline{H}_3$), 2.02–2.08 [m, 2H, $-O(CH_2)_2CH=CHC\underline{H}_2-$], 2.02–2.08 (m, 2H, $-C\underline{H}_2CH=CHCH_2OAc$), 2.33 (bq, J = 6.5 Hz, 2H, $-OCH_2C\underline{H}_2CH=CH-$), 3.35 (s, 3H, $C\underline{H}_3O-$), 3.38 (t, 2H, $CH_3OC\underline{H}_2-$), 4.50 (dq, J = 5.3, 15.4 Hz, 2H, $-CH=CHC\underline{H}_2OAc$), 5.42–5.50 [m, 2H, $-O(CH_2)_2C\underline{H}=C\underline{H}-$], 5.56 (dtt, J = 1.0, 5.5, 15.4 Hz, 1H, $-CH=C\underline{H}CH_2OAc$), 5.78 (dtt, J = 1.0, 6.5, 15.4 Hz, 1H, $-C\underline{H}=CHCH_2OAc$).

Estimation of Vapor Pressures

Principles. The relevant equations for determining vapor pressures by the GC method (P_{GC}) have been developed by Hamilton (1980). At a constant temperature, the vapor pressures of a test and of a reference compound (subscript t and r, respectively) are related by the ratio of their latent heats of vaporization:

$$\ln P_t = (H_t/H_r) \ln P_r + C \quad (1)$$

where H is the latent heat of vaporization and C is a constant. A similar equation has been developed for the GC retention times t:

$$\ln (t_t/t_r) = (1 - H_t/H_r) \ln P_r - C \quad (2)$$

Therefore, a plot of ln (t_t/t_r) versus ln P_r should have a slope $(1 - H_t/H_r)$ and an intercept $(-C)$. Equation 1 can then be used to determine the vapor pressure of the test compound at any temperature if the vapor pressure of the reference compound at that temperature is known.

Experimental Procedure. Samples were analyzed on a Hewlett Packard HP 5880 chromatograph equipped with a FID detector on a 2-m fused silica capillary column (cross-linked 5% methyl silicone, HP-1), 0.52 μm film thickness; splitless injection. The chromatography was operated isothermally at 10°C intervals from 110°C to 170°C with hydrogen flow 10 ml/min; C_{18} hydrocarbon was used as a reference compound. Vapor pressures of *n*-octadecane at different temperatures were calculated from the equation ln P(torr) $= A + B/T$ with parameters $A = 25.548$, and $B = -10165$ (Macknick and Prausnitz, 1979; Bidleman, 1984). Retention times were determined on an HP 3396A integrator. All runs were made at least in duplicate and average retention times were used for calculations. As recommended (Bidleman, 1984), long retention times of compounds producing unsymmetrical peaks at low temperatures were not taken at the peak maximum, but were estimated at the midpoint between the beginning and the end of the peak.

RESULTS AND DISCUSSION

Vapor Pressures of Compounds ***1–12.*** Since vapor pressures determined by using equations 1 and 2 (P_{GC}) may under- or overestimate the actual vapor pressures (P_L) depending on vapor pressure range and the GC column used (Bidleman, 1984), the P_{GC} estimates have to be generally calibrated to provide reasonable vapor pressure values. Accordingly, the previously developed (Koutek et al., 1992) calibration equation 3 was used to correct the P_{GC} values of all derivatives investigated.

$$\ln P_L(\text{Pa}) = 1.0126 \ln P_{GC} + 0.4440. \quad (3)$$

If the compound is solid (as is the carbamate **12,** with mp of 27°C) another problem arises, since its vapor pressure P_s will be lower than that of the subcooled liquid P_L by the factor of the fugacity ratio P_S/P_L. This has been previously shown (Mackay et al., 1982) to be expressible by

$$\ln (P_S/P_L) = -6.8 (T_M/298 - 1), \quad (4)$$

where T_M is the absolute melting point, and 6.8 is an empirical constant related to the entropy of fusion.

Corrected vapor pressures (P) based on equations 3 and 4 are summarized in Table 1 together with P_{REL} factors that were used to correct the EAG data.

TABLE 1. PARAMETERS OF EQUATION 2 AND VAPOR PRESSURES (25°C) OF COMPOUNDS **1–12**

Compound	H_i/H_r	C	$P^{(25)} \times 10^3$ (Pa) Eq. 1	$P^{(25)} \times 10^3$ (Pa) Eq. 3	P_{REL}[a]
1	1.1600	2.7241	0.930	1.328	1
2	1.1365	2.5654	1.188	1.701	1.281
3	1.1785	2.8208	0.789	1.124	0.846
4	1.1457	2.6409	1.065	1.523	1.147
5	1.2269	3.2673	0.423	0.598	0.450
6	1.2357	3.3202	0.388	0.548	0.413
7	1.2357	3.4286	0.353	0.498	0.375
8	1.2006	2.9845	0.618	0.878	0.661
9	1.1875	2.9191	0.692	0.984	0.741
10	1.1360	1.7715	2.633	3.808	2.867
11	1.2682	4.1164	0.155	0.216	0.163
12	1.3708	5.2620	0.034	0.044[b]	0.033

[a] $P_{REL} = P_{SUBSTANCE}/P_{E2,Z13\text{-}18:Ac}$.
[b] Corrected by using equation 4.

The results impressively demonstrate the importance of vapor pressure corrections in comparing the EAG data for compounds of different volatilities. Note that at 25°C the relative liquid–vapor concentration ratio for the pheromone component **1,** trifluoroacetate **10,** and carbamate **12** follows the order 1:1, 1:2.88, and 1:0.033, respectively. It means that the trifluoroacetate **10** is about 3 times more volatile while carbamate **12** about 30 times less volatile than the pheromone component **1.**

Electrophysiological Properties. Relative EAG activities of compounds **1–12** corresponding to 75% of the relative activity scale are summarized in Figure 1 together with the vapor pressure-corrected data. As expected, the sensitivity of the EAG data to vapor pressure corrections differs significantly with the type of compound. The largest differences are observed in the ester series **1, 10–12,** where the vapor pressure corrections may even cause changes in the activity order.

The following intermolecular forces in substrate–receptor interactions have been generally recognized: electrostatic attraction between charges and partial charges, steric repulsion, hydrophobic bonding, hydrogen bonding, and van der Waals attractions (dispersion, orientation, dipole–dipole, etc.) (Hansch and Leo, 1979). In addition, intramolecular forces in the substrate that affect its conformation may be important, depending on the conformational requirements of the receptor site (Hopfinger, 1980; Liljefors et al., 1985). In this context, the series

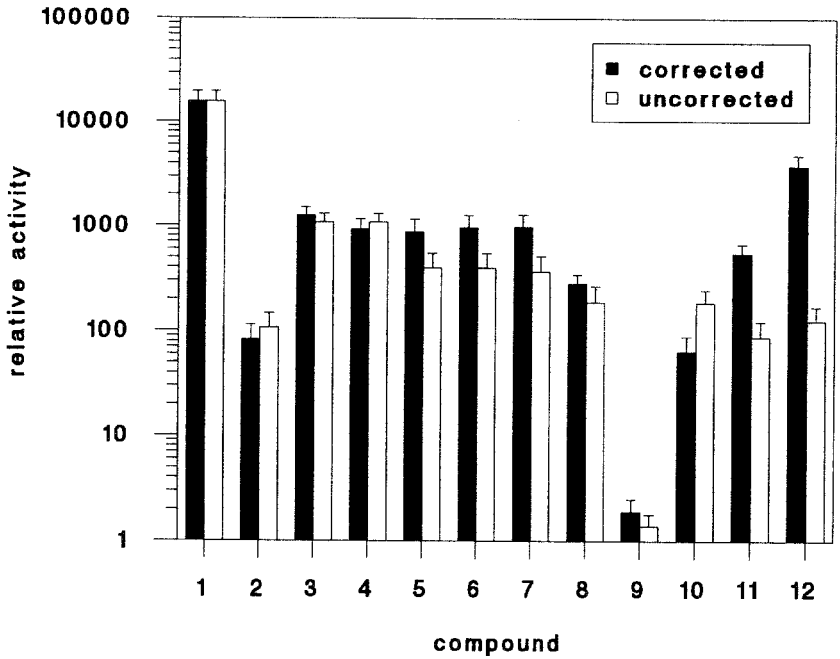


FIG. 1. Experimental EAG activities (+ SEM) for compounds **1–12.** A comparison of uncorrected and vapor pressure-corrected data. The activities are expressed as the reciprocal of the relative number of moles required to elicit the same receptor response.

of analogs **2–12** may be separated into two distinct types of compounds: (1) those where both the acetate and the terminal alkyl functions are constantly present and, as a consequence, conformational requirements may be presumed to be the main factor influencing biological activities (i.e., derivatives **1–7**); and (2) those containing an unchanged 2*E*,13*Z* double bond system, the structural differences taking place on the ester and terminal alkyl functions. Since the conformational properties of these compounds are virtually identical, other factors should be operative to rationalize the observed activity differences.

Inspection of Figure 1 shows that all analogs display a reduced activity compared to the natural pheromone component **1.** The EAG activity of geometrical isomers decreases in the relative ratio of about 188:15:10:1 for the *EZ*(**1**), *ZZ*(**3**), *ZE*(**4**) and *EE*(**2**) isomers, respectively. Thus, replacement of the 13*Z* double bond by its *E* equivalent in the parent acetate **1** seems to cause more drastic decrease in activity than does the *E* ⇒ *Z* change in position 2. Interestingly, some degree of similarity exists between the foregoing results and the EAG data of Tonini et al. (1986) on the leopard moth, *Zeuzera pyrina* L. (Lepidoptera: Cossidae). The *S. tipuliformis* and *Z. pyrina* species not only use

the *E,Z*-acetate **1** as a main pheromone component, but, of the analogs **2–4,** both species also elicit the largest response to the *Z,Z* isomer **3.**

The replacement of a $-CH_2-$ unit by an oxygen atom in the C_{16} and C_{17} positions resulted in a marked decrease of the biological activity. While the activity of the 17-oxa isomer **9** is the lowest of all the analogs investigated, introduction of oxygen into C_{16} position in derivative **8,** is far better tolerated. A linear relationship was observed (Figure 2) between logarithms of the relative activities and Hansch hydrophobic constants π for the terminal alkyl molecular parts $CH_3CH_2CH_2-$(1.55), CH_3CH_2O-(0.38) and CH_3OCH_2- (−0.78). The correlation equation corresponding to the linear least-squares fit is ln (*EAG*) = $3.832\pi + 3.868$ ($n = 3$, $r = 0.998$, SE = 0.413). These results seem to be in line with recent electrosensillography (ESG) findings on the turnip moth analogs (Jönsson et al., 1991b) that substituent size and hydrophobicity of the

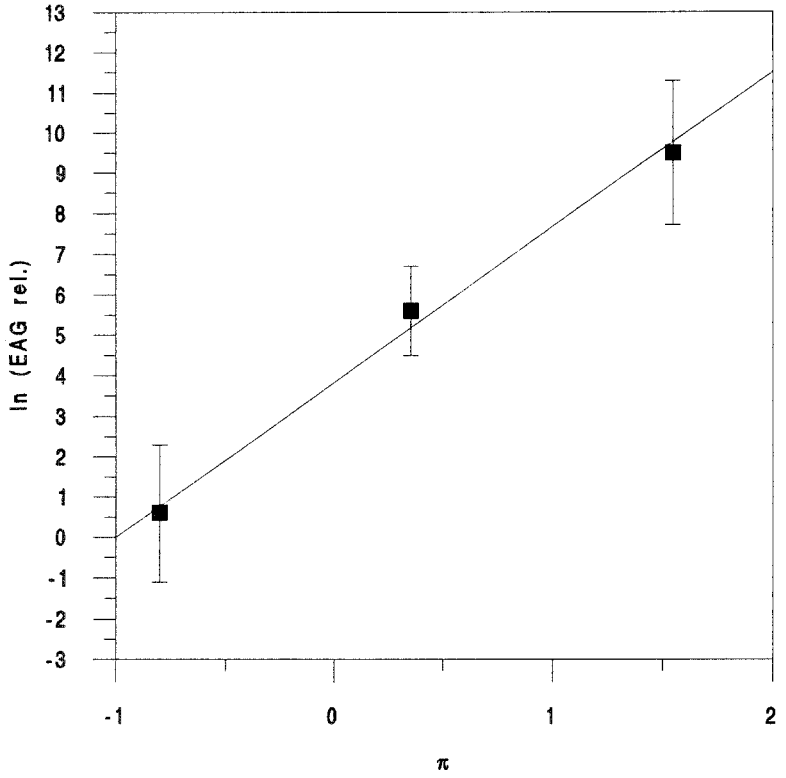


FIG. 2. Relationship between relative EAG activities of the compounds **1, 8,** and **9** and hydrophobic parameters for the terminal substituents $CH_3CH_2CH_2-$, CH_3CH_2O-, and CH_3OCH_2-, respectively. The uncertainty is given as 95% confidence interval.

terminal alkyl group are the most important factors influencing the activity. Because of the suggested bioisostericity of $CH_2 \Rightarrow O$ replacements, the activity of compounds **8** and **9** should be preferentially directed by hydrophobicity alone.

The last group of analogs included the trifluoroacetate **10,** pivalate **11,** and *N*-methyl carbamate **12,** which differ from the acetate **1** only in the R substituent of the ester moiety $-CH_2-O-CO-R$. The EAG activity of these compounds decreases in the order $CH_3 > NHCH_3 > C(CH_3)_3 > CF_3$. Trifluoroacetate **10** showed only less than 1% of the activity elicited by the parent acetate **1.** No clear trend in activity is observed when single parameters describing steric demand (E_s, STERIMOL parameters), liphophilicity, or electronic (F, R) effects are used as substituent descriptors for the ester series. Evidently, one factor is not sufficient to reproduce the EAG data. To rationalize the experimental data, polar effect and size of the substituent R have to be combined. Thus, F (Hansch and Leo, 1979) and L (Verloop et al., 1976) parameters representing the inductive effect and length of the substituents were simultaneously used to express the structural effects numerically. The variation of activity of ester analogs **1, 10–12** is then given by equation $\ln (EAG) = -10.301F - 3.287L + 18.954$ ($N = 4$, $R^2 = 98.22\%$, SE = 0.260). This equation predicts that a substituent with a smaller electron-withdrawing effect and smaller steric demand should have a greater EAG activity. Using this equation, relative EAG activities of 13500, 350, and 65, respectively, are predicted for the acetate **1,** (*E*,*Z*)-2,13-octadecadien-1-yl propanoate and trifluoroacetate **10.** It is interesting to note that the same hierarchical order has been found (Liljefors et al., 1984) for ESG activity of (*Z*)-7-dodecenyl acetate and its propanoate and trifluoroacetate analogs on the turnip moth *Agrotis segetum.* Moreover, in both cases the differences (dE) also follow the same trend, i.e. dE(acetate − propanoate) >> dE(propanoate − trifluoroacetate). Even though a more detailed discussion of these results is precluded owing to the incompatibility of data presentation as well as limited number of points, it seems that an activity order acetate > propanoate > trifluoroacetate could be generally valid for a variety of acetate-responding species (cf. also behavioral studies of Camps et al., 1988 on the processionary moth *Thaumetopoea pityocampa*).

Among the ester analogs investigated, the carbamate **12** deserves a special comment. This derivative belongs to the group of carbamones, as the *N*-methyl carbamate based pheromone analogs have been called. One such carbamone, *Z*9–14:Nmc (*N*-methyl carbamate) was reported as a chemically reactive pheromone analog capable of sensory disruption of the *Heliothis virescens* males (Albans et al., 1984). In contrast, however, no disruption of the perception of *Z*11–16:Ac in *Mamestra brassicae* by the carbamone *Z*11–16:Nmc has been found, in spite of the fact that this carbamate analog elicited an EAG response only slightly less than the natural pheromone component *Z*11–16:Ac (Prestwich, 1987). In view of these findings, the relatively high activity of **12** suggests (but does not prove) a biological activity in the behavioral experiments.

To conclude, we have shown that the CCM males possess receptors capable of responding to analogs of the acetate **1.** The results on analogs only changed in the terminal alkyl group indicate that hydrophobicity plays an important role in the structure–activity relationships. On the other hand, when the analogs are modified on the ester group, the EAG activity order suggests that inductive and steric effects are of primary importance. Single-cell studies will be necessary to provide information whether the analogs interact with the same receptor as **1.** Although the use of a more complete basis set would be needed to supply a more correct representation of the structure–activity relationship, the present data qualitatively agree with the generally accepted view for monoenic acetates as it has been derived from the single-cell measurements.

Significant differences between the vapor pressure-corrected and uncorrected EAG activity data for some of the analogs investigated show that we should be cautious about the validity of conclusions formerly drawn from structure–activity studies without considering different volatilities of the compounds.

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