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ANALOGS OF SEX PHEROMONE OF PROCESSIONARY MOTH, *Thaumetopoea pityocampa*:[1] SYNTHESIS AND BIOLOGICAL ACTIVITY

FRANCISCO CAMPS,[2] GEMMA FABRIÀS,[2] VICENS GASOL,[2] ANGEL GUERRERO,[2] RODOLFO HERNÁNDEZ,[3] and RAMÓN MONTOYA[3]

[2]*Departamento de Quìmica Orgánica Biológica, C.I.D. (CSIC) Jorge Girona Salgado, 18-26. 08034-Barcelona, Spain*

[3]*Sección de Equilibrios Biológicos, ICONA Gran Via de San Francisco, 35-41. 28005-Madrid, Spain*

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Abstract—The synthesis and biological activity of some analogs of (*Z*)-13-hexadecen-11-ynyl acetate **1**, the major component of the sex pheromone of the processionary moth *Thaumetopoea pityocampa* is described. The analogs have been formally derived by structural modification of the enyne and acetate functions of the parent compound **1**. In field tests, trifluoroacetate ester **16** and the analog, **11**, with fluorine substitution at the olefin site, decreased the pheromone action, whereas epoxy derivative, **10**, from epoxidation of the olefin moiety in **1**, and propionate ester **15** gave synergistic activity. The formate **14** had a variable effect according to the composition of the lure. Formal reduction of the enyne to give the acetylene **2** was found to retain activity. Alcohols **12** and **13**, resulting from hydrolysis of the enyne **1** and acetylene **2**, respectively, inhibited the action of their parent compounds.

Key Words—Sex pheromone, inhibition, synergism, processionary moth, *Thaumetopoea pityocampa*, Lepidoptera, Thaumetopoeidae.

INTRODUCTION

It is generally accepted that in the perception process of insect sex pheromones, there are very strict structural requirements for successful interaction between

[1] Lepidoptera, Thaumetopoeidae.

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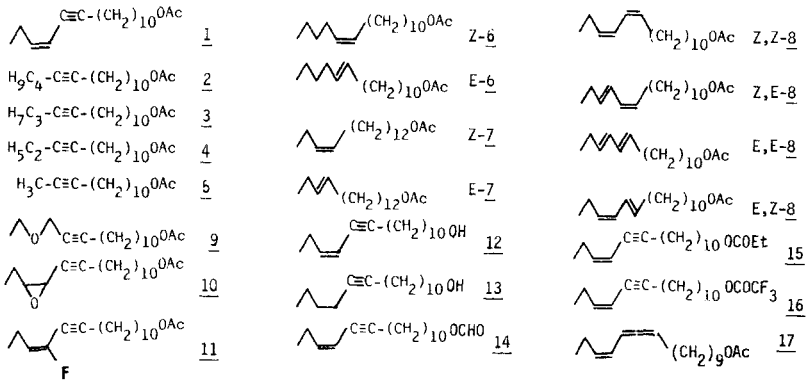
substrate and the receptor cells in the dendritic membrane of the antenna. This interaction may involve electrostatic, hydrophilic, hydrophobic, van der Waals forces or a hydrogen bonding mechanism and can be modulated by flexible receptor proteins, which can adopt the required conformational changes to achieve a successful recognition of the substrate molecule.

In this context, the knowledge of the sex pheromone critical molecular parts may be essential for a better understanding of the overall attractant perception process, and for the establishment of structure–activity relationships (Priesner, 1979; Liljefors et al., 1984) to help in the design of new synergists and inhibitors, capable of either increasing or decreasing the biological activity of the natural pheromone (Roelofs and Comeau, 1971).

In this paper, we report on the synthesis and biological activity of a variety of compounds structurally related to (*Z*)-13-hexadecen-11-ynyl acetate **1** (pityolure), the major component of the sex pheromone of the processionary moth *Thaumetopoea pityocampa* (Denis and Schiff.) (Guerrero et al., 1981; Camps et al., 1981a,b, 1983, 1987a; Cuevas et al., 1983; Michelot et al., 1982; Shani et al., 1983). These compounds, **2–17** (Scheme 1) proceed from modifications on the three putative key molecular parts of the parent molecule **1**, the double and the triple bonds and the acetate group, which may be directly involved in the interaction process with the receptor.



SCHEME 1.

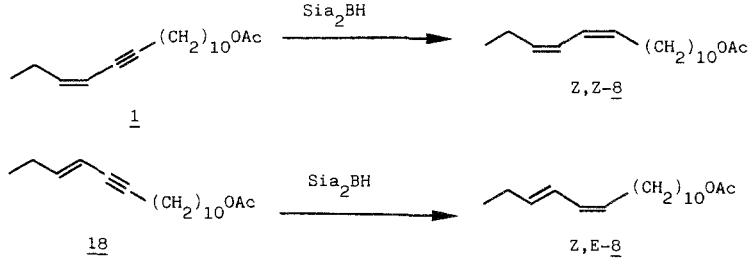
Thus, acetylenic derivatives **2–5** would result from saturation of the double bond and shortening of the chain length of the nonfunctionalized part of the sex pheromone component **1**. Likewise, partial reduction of the enyne moiety would lead to dienic compounds **8**, from which olefins **6** and **7** could be derived. On the other hand, whereas in oxa-analog **9** one of the olefinic carbons has been replaced by an oxygen atom, in the epoxy derivative **10** the double bond has been transformed into an oxirane ring. In fluorinated analog **11**, a fluorine atom

has been substituted for a vinyl hydrogen, whereas alcohols **12** and **13** would, obviously, derive from the corresponding esters **1** and **2**. Finally, in esters **14**, **15**, and **16**, the acetate moiety has been replaced by the formate, propionate, and trifluoroacetate groups, respectively.

METHODS AND MATERIALS

Boiling points are uncorrected. IR spectra were recorded in CCl_4 solution on Perkin Elmer 257 and 399B grating spectrometers [^{1}H]NMR spectra were determined in $CDCl_3$ solution on a Bruker WP80SY or on a Varian XL200 spectrometer, operating at 80 and 200 MHz, respectively, and absorptions are expressed in δ scale relative to TMS. [^{13}C]NMR spectra were recorded on a Bruker WP80SY instrument at 20.15 MHz in $CDCl_3$ solution, and the values are expressed in δ scale relative to TMS. GLC analyses were performed on Carlo Erba models 2350 and 4130 equipped with a FID detector, using 3% OV-101 glass column 2 m × 3 mm ID on Chromosorb W (nitrogen as carrier gas), or a fused silica capillary column SE-54 50 m × 0.32 mm ID (hydrogen as carrier gas).

Reactions requiring anhydrous and oxygen-free conditions were performed under dried inert atmosphere (N_2 or Ar). Anhydrous solvents were prepared as follows: tetrahydrofuran (THF) by distillation from Na/benzophenone, diethyl ether from lithium aluminium hydride (LAH), CCl_4 from P_2O_5, pyridine from KOH, and hexamethylphosphoric triamide (HMPT) from CaH_2.



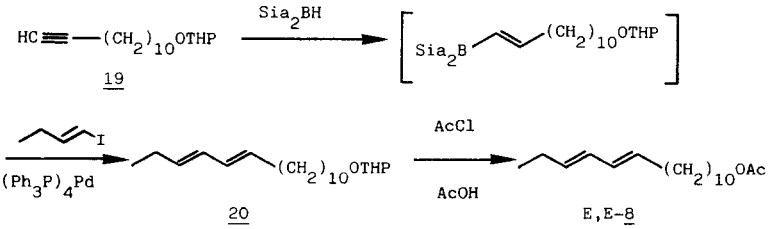
SCHEME 2.

Chemicals. Acetylenic acetates **2–5** were prepared by alkylation of the corresponding tetrahydropyranyloxy acetylides in HMPT (Brattesani and Heathcock, 1973) followed by hydrolysis and acetylation. Monounsaturated derivatives *Z*-**6**, *Z*-**7**, *E*-**6**, and *E*-**7** have been prepared by stereoselective reduction of the parent acetylenic compounds to the *Z* (H_2/Pd-C) or to the *E* isomer (Na/NH_3). The acetylenic precursor for preparation of compounds **7** was obtained by triple bond migration of tetradec-11-yn-1-ol with potassium

3-aminopropylamide (KAPA) in 1,3-propylenediamine (Brown and Yamashita, 1975), followed by hydroxyl protection as the tetrahydropyranyl derivative, alkylation of the resulting terminal acetylene with ethyl bromide, hydrolysis, and acetylation. The epoxyacetate **10**, which was prepared by epoxidation of the natural compound **1**, was subsequently transformed into fluorinated analog **11**, with a 2:1 *E*:*Z* isomer ratio, by a route previously described by us (Camps et al., 1986). Formate **14**, propionate **15**, and trifluoroacetate **16** have been prepared by esterification of the parent alcohol **12**. On the other hand, eneallene **17** was kindly supplied by Dr. Jahyo Kang (Sogang University, Seoul, South Korea).

(Z,Z)-*11,13-Hexadecadienyl Acetate* (Z,Z)-*8* (Scheme 2). In a two-neck 25-ml round-bottomed flask was placed 0.065 ml (0.5 mmol) of a 8 M BH_3 · THF complex. To this solution, previously cooled to 0°C, was added, under N_2, 0.070 g (1 mmol) of 2-methyl-2-butene. The resulting mixture was stirred for 2 hr at room temperature and cooled again to 0°C. Then, 0.115 g (0.42 mmol) of (*Z*)-13-hexadecen-11-ynyl acetate **1** in 2 ml of anh. ether was added. Stirring was kept at room temperature for 4 hr, then 0.3 ml of glacial acetic acid was added and the resulting solution further stirred for 12 hr at 30–35°C. Oxidation was carried out by stirring for 30 min in the presence of 1 ml of 6 N NaOH and 0.25 ml of 30% hydrogen peroxide, keeping the temperature below 40°C. The reaction mixture was poured into ice and extracted with hexane (4 × 8 ml). The combined organic layers were washed with brine and dried ($MgSO_4$) to yield, after evaporation of the solvent, 0.116 g of an oil, which was purified by column chromatography on silica gel, eluting with hexane–ethyl acetate 95:5, to afford pure (*Z*,*Z*)-**8** in 76% yield (isomeric purity higher than 99% by GLC analysis on capillary column).

Anal.: Calcd. for $C_{18}H_{32}O_2$: C, 77.14; H, 11.42. Found: C, 77.15; H, 11.72. IR: ν 2960, 2930, 1860, 1745, 1240 cm^{-1}. [^{1}H]NMR: δ 1.00 (t, J = 7.5 Hz, 3H, $C\underline{H}_3CH_2$), 1.28 (b, 14H, $CH_2C\underline{H}_2CH_2$), 1.62 (c, 2H, $C\underline{H}_2CH_2O$), 2.05 (s, 3H, $C\underline{H}_3CO$), 2.16 (c, 4H, $C\underline{H}_2C{=}C$), 4.05 (t, J = 6.7 Hz, 2H, $C\underline{H}_2O$), 5.44 (c, 2H, $CH_2C\underline{H}{=}$), 6.24 (c, 2H, ${=}C\underline{H}\text{-}C\underline{H}{=}$). [^{13}C]NMR: δ 64.7 (C-1), 28.7–29.7 (C-2 and C-4 to C-9), 26.0 (C-3), 27.5 (C-10), 133.6 (C-11), 123.2, 123.6 (C-12 and C-13), 132.1 (C-14), 20.9 (C-15 and C-1′), 14.2 (C-16), 171.0 (CO).



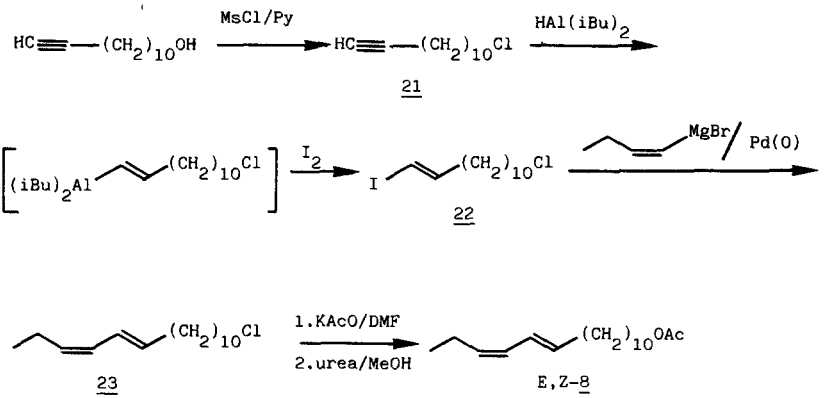
SCHEME 3.

(Z,E)-*11,13-Hexadecadienyl Acetate* (Z,E)-***8*** (Scheme 2). The same procedure described above for compound ***Z,Z*-8** was applied, using compound **18,** the *E* isomer of **1**, previously prepared by coupling of (*E*)-1-iodo-1-butene with 11-dodecynyl acetate in the presence of tetrakis(triphenylphosphine)palladium (Michelot et al., 1982). Thus, starting from 88 mg (0.31 mmol) of **18**, 72 mg (82%) of dienic acetate (*Z,E*)-**8** was obtained, after purification by column chromatography on silica gel, eluting with hexane–ethyl acetate 95:5. Compound (*Z,E*)-**8** had a 98:2 *Z,E*:*E,E* isomeric purity by GLC analysis on capillary column.

Anal.: Calcd. for $C_{18}H_{32}O_2$: C, 77.14; H, 11.42. Found: C, 77.17; H, 11.63. IR: ν 2960, 2930, 1745, 1240, 980 cm^{-1}. [^{1}H]NMR: δ 1.01 (t, J = 7.4 Hz, 3H, $C\underline{H}_3CH_2$), 1.29 (b, 14H, $CH_2C\underline{H}_2C\underline{H}_2$), 1.61 (c, 2H, $C\underline{H}_2CH_2O$), 2.05 (s, 3H, $C\underline{H}_3CO$), 2.16 (c, 4H, $C\underline{H}_2C{=}$), 4.05 (t, J = 6.7 Hz, 2H, $C\underline{H}_2O$), 5.31 (dt, J = 10.8 and 7.3 Hz, 1H, H-14), 5.70 (dt, J = 15 and 6.7 Hz, 1H, H-11), 5.95 (t, J = 10.8 Hz, 1H, H-13), 6.31 (dd, J = 15.1 and 10.9 Hz, 1H, H-12). [^{13}C]NMR: δ 64.5 (C-1), 28.6–29.7 (C-2 and C-4 to C-9), 25.8 (C-3), 27.6 (C-10), 130.0 (C-11), 128.6 (C-12), 124.7 (C-13), 136.0 (C-14), 25.8 (C-15), 13.6 (C-16), 20.9 (C-1′), 170.8 (CO).

(E,E)-*11,12-Hexadecadienyl Acetate* (E,E)-***8*** (Scheme 3). In a three-neck round-bottomed flask was placed, at 0°C under N_2, a mixture of 0.178 ml (1.52 mmol) of a 8 M $BH_3 \cdot S(CH_3)_2$ solution and 0.210 g (3 mmol) of 2-methyl-2-butene. The resulting solution was stirred at room temperature for 2 hr and then a solution of 0.353 g (1.27 mmol) of 2-(11-dodecynyloxy)tetrahydropyran **19** in 2 ml of anh. THF was added. The mixture was stirred for 2 hr to complete the formation of the intermediate vinylborane. After this time, a solution of 8 mg (1% molar) of tetrakis(triphenylphosphine)palladium in 1 ml of anh. THF, previously stirred at room temperature for 30 min on the dark and under N_2, was added along with 0.231 g (1.27 mmol) of (*E*)-1-iodo-1-butene (Michelot et al., 1982). The mixture was stirred for 2 hr more at room temperature and treated with a solution of 0.1 g of NaOH in 1.2 ml of deoxygenated water for 14 hr at 70°C. Oxidation was carried out by addition at 0°C of 1.2 ml of 6.5 N NaOH and 0.4 ml of 30% H_2O_2, followed by stirring for one additional hour at room temperature. The reaction mixture was poured into ice and extracted with hexane (5× 10 ml). The combined organic layers were washed with brine and dried ($MgSO_4$) to afford, after evaporation of the solvent, 0.36 g of the expected THP-protected dienic alcohol **20**, which was directly acetylated by treatment with 0.25 ml of acetyl chloride and 1 ml of acetic acid in 2 ml of CCl_4 under reflux for 7 hr. Then, the reaction mixture was cooled, and after addition of solid $NaHCO_3$, poured into ice and extracted with hexane (5× 10 ml). The organic layers were washed with brine and dried ($MgSO_4$). Removal of the solvent and purification by column chromatography on silica gel, eluting with hexane–ethyl acetate 95:5, afforded 0.155 g (43%) of compound (*E,E*)-**8**, isomerically pure (99%) by GLC analysis.

Anal.: Calcd. for $C_{18}H_{32}O_2$: C, 77.14; H, 11.42. Found: C, 77.04; H, 11.30. IR: ν 2960, 2930, 2860, 1745, 1240, 990 cm^{-1}. [^{1}H]NMR: δ 0.99 (t, J = 7.4 Hz, 3H, $C\underline{H}_3CH_2$), 1.29 (b, 14H, $CH_2C\underline{H}_2CH_2$), 1.62 (c, 2H, $C\underline{H}_2CH_2O$), 2.04 (s, 3H, $C\underline{H}_3CO$), 2.06 (c, 4H, $C\underline{H}_2C{=}$), 4.05 (t, J = 6.7 Hz, 2H, $C\underline{H}_2O$), 5.58 (c, 2H, $CH_2C\underline{H}{=}$), 6.00 (c, 2H, $=C\underline{H}\text{-}C\underline{H}{=}$). [^{13}C]NMR: δ 64.5 (C-1), 28.5–29.3 (C-2 and C-4 to C-9), 25.8 (C-3), 32.5 (C-10), 132.2 (C-11), 130.5 (C-12), 130.3 (C-13), 132.0 (C-14), 25.4 (C-15), 13.5 (C-16), 21.0 (C-1′), 170.9 (CO).



SCHEME 4.

12-Chloro-1-dodecyne ***21***. In a three-neck round-bottomed flask provided with a magnetic stirrer, N_2 inlet, septum, reflux condenser, and calcium chloride tube were placed 1.13 g (6.2 mmol) of 11-dodecyn-1-ol and 0.54 g (6.8 mmol) of anh. pyridine in 3 ml of anh. dimethylformamide (DMF). To this solution was added 0.52 ml (6.76 mmol) of methanesulfonyl chloride, and the reaction mixture was heated at 75°C for 16 hr. The resulting solution was poured into ice-water and extracted with hexane (5× 20 ml). The combined organic layers were washed with brine and dried ($MgSO_4$). Removal of the solvent left a residue which was distilled (bp 60–65°C/0.3 torr) to yield 1.0 g (88%) of pure chloroalkyne **21**.

Anal.: Calcd. for $C_{12}H_{21}Cl$: C, 72.07; H, 10.58. Found: C, 72.15; H, 11.00. [^{1}H]NMR: δ 1.32 (b, 14H, $CH_2C\underline{H}_2CH_2$), 1.79 (c, 2H, $C\underline{H}_2CH_2Cl$), 1.94 (t, J = 2.5 Hz, 1H, $\underline{H}C{\equiv}$), 2.16 (c, 2H, $C\underline{H}_2C{\equiv}$), 3.55 (t, J = 6.6 Hz, 2H, $C\underline{H}_2Cl$). [^{13}C]NMR: δ 68.0 (C-1), 84.4 (C-2), 18.2 (C-3), 28.4–29.2 (C-4 to C-9), 26.8 (C-10), 32.5 (C-11), 44.8 (C-12).

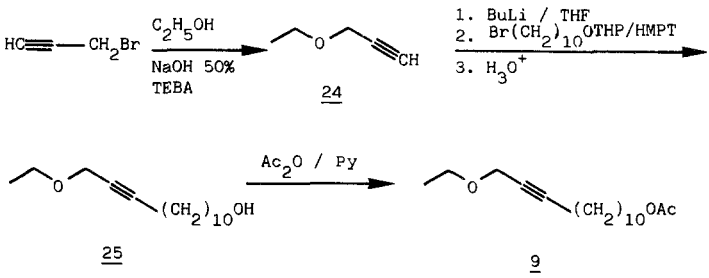
(E)-*12-Chloro-1-iodo-1-dodecene* ***22***. To a solution of 1.4 g (7 mmol) of alkyne **21** in 7 ml of anh. hexane was added dropwise, under N_2, 7 ml (7 mmol)

of a 1 M diisobutylaluminiumhydride (DIBAH) soln. in hexane. The reaction mixture was stirred at 50°C for 4 hr, cooled to −40°C, and subsequently treated with 1.77 g (7 mmol) of iodine in 7 ml of anh. THF. Stirring was continued for 1 hr at room temperature. The mixture was cooled to −10°C and 5 ml of 20% H_2SO_4 was slowly added. After pouring into ice and extraction with hexane (5× 20 ml), the combined organic layers were washed with 5% $NaHSO_3$ and brine and dried ($MgSO_4$). Evaporation of the solvent yielded a crude, which was chromatographically purified (silica gel) to afford 1.42 g (62%) of compound **22**, isomerically pure (>99%) by GLC analysis on a capillary column.

Anal.: Calcd. for $C_{12}H_{22}ClI$: C, 43.94; H, 6.76, Cl, 10,81; I, 38.69. Found: C, 43.77; H, 7.09; Cl, 10.79; I, 38.66. IR: ν 2930, 2860, 1605, 950 cm^{-1}. [^{1}H]NMR: δ 1.31 (b, 14H, $CH_2\underline{CH_2}\underline{CH_2}$), 1.82 (c, 2H, $\underline{CH_2}CH_2Cl$), 2.05 (c, 2H, $\underline{CH_2}C=$), 3.55 (t, J = 6.6 Hz, 2H, $\underline{CH_2}Cl$), 5.97 (dt, J = 14.4 and 1.6 Hz, 1H, $=\underline{CH}I$), 6.52 (dt, J = 14.5 and 7.4 Hz, 1H, $=\underline{CH}CH_2$). [^{13}C]NMR: δ 74.2 (C-1), 146.7 (C-2), 36.0 (C-3), 28.3–29.4 (C-4 to C-9), 26.9 (C-10), 32.6 (C-11), 45.0 (C-12).

(E,Z)-*11,13-Hexadecadienyl Acetate* (E,Z)-**8** (Scheme 4). A THF solution of the Grignard reagent of (*Z*)-bromo-1-butene, obtained as previously described (Norris, 1959), was prepared from 0.4 g (3 mmol) of the alkene and 86 mg (3.6 mmol) of magnesium. The organomagnesium reagent was slowly added, via syringe, to a solution of 0.329 g (1 mmol) of compound **22** and 0.173 g (0.15 mmol) of tetrakis(triphenylphosphine)palladium in 7 ml of anh. THF. The resulting mixture was stirred at room temperature for 2 hr, poured into ice-water and extracted with hexane (5× 10 ml). After conventional work-up, 0.170 g of crude (*E,Z*)-11,13-1-chlorohexadecadiene **23** was obtained, which was directly treated with 0.490 g (15 mmol) of KOAc in 10 ml of DMF at 120°C for 90 min. The reaction mixture was quenched and worked up as usual to provide 0.162 g of a crude, which was purified by column chromatography on silica gel, eluting with hexane–ethyl acetate 94:6, to furnish 0.146 g (52% from **22**) of diene acetate (*E,Z*)-**8**, of isomeric purity *E,Z*:*E,E* 82:18 on GLC analysis. Removal of the contaminating *E,E* isomer was achieved by treatment with a saturated solution of urea in methanol to afford compound (*E,Z*)-**8** in 97% stereomeric purity.

Anal.: Calcd. for $C_{18}H_{32}O_2$: C, 77.14; H, 11.42. Found: C, 77.42; H, 11.36. IR: ν 2980, 2930, 1745, 1240 cm^{-1}. [^{1}H]NMR: δ 0.99 (t, J = 7.5 Hz, 3H, $\underline{CH_3}CH_2$), 1.28 (b, 14H, $CH_2\underline{CH_2}\underline{CH_2}$), 1.6 (c, 2H, $\underline{CH_2}CH_2O$), 2.05 (s, 3H, $\underline{CH_3}CO$), 2.12 (c, 4H, $\underline{CH_2}C=$), 4.05 (t, J = 6.7 Hz, 2H, $\underline{CH_2}O$), 5.30 (dt, J = 10.7 and 7.3 Hz, 1H, H-14), 5.6 (dt, J = 14.9 and 7.2 Hz, 1H, H-11), 5.92 (t, J = 10.7 Hz, 1H, H-13), 6.33 (dd, J = 14.9 and 10.7 Hz, 1H, H-12). [^{13}C]NMR: δ 64.5 (C-1), 28.6–29.4 (C-2 and C-4 to C-9), 25.8 (C-3), 32.8 (C-10), 134.6 (C-11), 125.5 (C-12), 128.0 (C-13), 131.5 (C-14), 20.9 (C-15), 14.2 (C-16), 20.9 (C-1′), 170.8 (CO).



SCHEME 5.

Ethyl 2-propynyl Ether ***24***. To a mixture of 5.6 ml (0.1 mol) of ethanol, 40 ml (0.5 mol) of a 50% solution of NaOH and 1.14 g (5 mmol) of benzyltriethylammonium chloride, previously cooled to 0°C, was added 13 g (0.11 mol) of freshly distilled propargyl bromide. Stirring was continued for 2 hr at room temperature, the organic layer was decanted and washed with brine, dried ($MgSO_4$), and distilled to yield 6.0 g (71%) of pure acetylene **24** (bp 58–60°C/170 torr). IR: ν 3300, 2970, 2860, 1100 cm^{-1}. [^{1}H]NMR: δ 1.19 (t, J = 7 Hz, 3H, $C\underline{H}_3$), 2.37 (t, J = 2.4 Hz, 1H, $\underline{H}C\equiv$), 3.50 (q, J = 6.9 Hz, 2H, $CH_3C\underline{H}_2O$), 4.10 (d, J = 2.4 Hz, 2H, $OC\underline{H}_2C\equiv$). [^{13}C]NMR: δ 62.6 (C-1), 95.6 (C-2), 57.6 (C-3), 65.4 (C-4), 14.7 (C-5).

14-Oxa-hexadec-11-ynol ***25***. To a solution of 0.504 g (16 mmol) of ether **24** in 10 ml of anh. THF at −10°C under Ar, was added 5 ml (5mmol) of a 1 M *n*-BuLi soln. in hexane. The resulting solution was stirred for 15 min and then 1.44 g (4.5 mmol) of 2-(10-bromodecyloxy)tetrahydropyran in 5 ml of anh. THF was added dropwise. The stirring was continued for 4 hr and the reaction mixture poured into ice and extracted with hexane (5× 20 ml). The combined organic layers were washed with brine and dried ($MgSO_4$) to give, after evaporation of the solvent, an oily residue which was treated with pyridinium tosylate (60 mg) in 20 ml of ethanol for 6 hr under reflux. The reaction mixture was worked up in the usual manner to yield 1.08 g (70%) of alcohol **25**, pure enough to be used in the next step without further purification. IR: ν 3600, 3300, 2920, 2850, 1090, 860 cm^{-1}. [^{1}H]NMR: δ 1.22 (t, J = 7 Hz, 3H, $C\underline{H}_3$), 1.40 (b, 16H, $CH_2C\underline{H}_2CH_2$), 2.20 (c, 2H, $CC\underline{H}_2C\equiv$), 3.55 (q, J = 7 Hz, 2H, $CH_3C\underline{H}_2O$), 3.65 (t, J = 6.7 Hz, 2H, $C\underline{H}O_2OH$), 4.12 (t, J = 2.2 Hz, 2H, $OC\underline{H}_2C\equiv$).

14-Oxa-hexadec-11-ynyl Acetate ***9*** (Scheme 5). Acetylation of alcohol **25** was carried out under conventional conditions (acetic anhydride/pyridine). Thus, starting from 0.856 g (3.2 mmol) of alcohol **25**, acetic anhydride (6 ml) and anh. pyridine (6 ml), pure acetate **9** (0.733 g, 83%) was obtained after purification on silica gel, eluting with hexane–ethyl acetate 90:10. Anal.: Calcd. for $C_{17}H_{30}O_3$:

C, 72.28; H, 10.60. Found: C, 72.20; H, 10.65. IR: ν 2930, 2850, 1740, 1240, 1090, 910 cm^{-1}. [^{1}H]NMR: δ 1.20 (t, J = 7.2 Hz, 3H, $C\underline{H}_3CH_2$), 1.38 (b, 16H, $CH_2C\underline{H}_2CH_2$), 2.05 (s, 3H, $C\underline{H}_3CO$), 2.20 (c, 2H, $CC\underline{H}_2C\equiv$), 3.55 (q, J = 7.2 Hz, 2H, $CH_3C\underline{H}_2O$), 4.05 (t, J = 7 Hz, 2H, $C\underline{H}_2OAc$), 4.11 (t, J = 2.1 Hz, 2H, $OC\underline{H}_2C\equiv$). [^{13}C]NMR: δ 64.4 (C-1), 28.5–29.3 (C-2 and C-4 to C-9), 25.8 (C-3), 18.6 (C-10), 76.2 (C-11), 86.5 (C-12), 58.2 (C-13), 65.0 (C-15), 14.8 (C-16), 20.7 (C-1′), 170.8 (C-0).

EAG Bioassays. The electroantennogram activity of the test compounds was determined on an EAG set-up with a new non-operator-dependent sample injection system (Guerrero et al., 1986).

Field Tests. The required amount of test compound in each bait was mixed with 2.5 mg of paraffin wax to slow down the release rate in the field (Cuevas et al., 1983), and dissolved in 1 ml of nanograde hexane. The solutions were transferred into closed polyethylene vials (3 × 1.1 cm ID) which were used as dispensers. Field trials were carried out in Mora de Rubielos (Teruel) and Cieza (Murcia) from 1984 to 1986. Traps used throughout the test seasons were "dry" (no glue added) and specially designed for processionary moth catches (Montoya, 1984). Traps were hung on trees at a height of 1.7–2.0 m above ground and those located in the same parcel were spaced 50 m apart. Minimum distance between parcels was 150 m apart. They were set out in statistically randomized blocks and revised and rotated every day. Ten traps were generally used for each formulation. In each parcel, the number of catches of the different formulations was compared with that of pityolure. Trap catches were subjected to a square-root transformation followed by analysis of variance, and the data were analyzed statistically for significance according to Duncan's multiple-range test. When appropriate, Student's t test was used to assess the significance of differences between mean numbers of catches within the same parcel.

RESULTS AND DISCUSSION

As shown in Table 1, the biological activity of fluorinated analog **11** was studied in 1984 and 1985 field trials. While traps baited with compound **11** alone were only slightly active (parcel I, test 1), lures with mixtures of **11** and the natural pheromone **1** caught variable number of males, depending on their composition. Remarkably, when a 3:1 ratio of **11**:**1** was used, an approximately 50% inhibitory effect on the activity of the pheromone was observed (parcel III, tests 1 and 2), whereas the results were not statistically significant when the 1:1 ratio was utilized.

The effects exhibited by propargylic ether **9**, epoxide **10**, formate **14**, propionate **15**, and allene **17** were studied in other experiments carried out in 1984 and 1985 (Table 2). Significant synergistic effects appeared to be displayed by

TABLE 1. CAPTURE OF *Thaumetopoea pityocampa* MALES WITH BLENDS OF FLUORINATED COMPOUND **11** AND PITYOLURE **1** IN COMPARISON WITH NATURAL COMPOUND **1**

		Composition of the lure (mg)		
Test[a]	Parcel	Fluorinated analog **11**	Pityolure **1**	Mean catch/trap/week[b]
1	I	1.0	—	12.5a
			1.0	58.4a
	II	0.9	0.1	4.5b
			1.0	17.6b
	III	0.75	0.25	15.2c
			1.0	40.2c
	IV	0.5	0.5	11.9d
			1.0	24.2d
	V	0.25	0.75	17.1NS
			1.0	23.2NS
	VI	0.1	0.9	23.8NS
			1.0	24.9NS
2	I	0.1	1.0	24.2NS
			1.0	23.2NS
	II	1.0	1.0	10.9NS
			1.0	11.7NS
	III	3.0	1.0	12.4a
			1.0	24.3a

[a] Tests 1 and 2 were conducted in Mora de Rubielos (Teruel) in 1984 and 1985, respectively.
[b] Means within parcel followed by the same letter are significant at $P < 0.05$, Student's t test. NS = nonsignificant. Ten replicates per treatment.

ether **9** and epoxide **10** when mixed with pityolure **1** in 1:10, 1:1 and 2.3:1 ratios (parcel I, test 1, and parcels I and II, test 2 for compound **9**; and parcel III, test 1, and parcels VII and VIII, test 2, for compound **10**). On the other hand, formate **14** behaved in a strikingly different way, depending upon the composition of the bait. Thus, when this compound was mixed with pityolure in a 1:10 ratio, it showed a significant synergistic effect (parcel IV, test 2), whereas at higher ratios (2.3:1 parcel II, test 1, and 10:1 parcel VI, test 2) a marked decrease in catches was observed. Almost no effect was shown in baits charged with 1 mg of both compounds (parcel V, test 2).

Previous authors have also reported on the synergistic effect exhibited by ethers obtained by replacing an olefinic carbon by oxygen in the pheromone structure of a different species, such as the red-banded leaf roller moth *Argyrotaenia velutinana* (Roelofs and Comeau, 1971). In this case, both oxygen analogs exhibited similar activities. Unfortunately, in the present study, we

TABLE 2. EFFICIENCY OF LURES BAITED WITH COMPOUNDS **9**, **10**, **14**, **15**, AND **17** IN COMBINATION WITH PITYOLURE **1**

		Composition of bait (mg)						
Test[a]	Parcel	Ether **9**	Epoxide **10**	Formate **14**	Propionate **15**	Allene **17**	Pityolure **1**	Mean/catch/ trap/week[b]
1	I	0.7					0.3	19.32a
							0.3	10.92a
	II			0.7			0.3	8.80b
							0.3	22.12b
	III		0.7				0.3	15.12c
							0.3	6.80c
2	I	0.1					1.0	36.86a
							1.0	29.36a
	II	1.0					1.0	13.11b
							1.0	9.82b
	III[c]					1.0	1.0	14.2NS
							1.0	18.63NS
	IV			0.1			1.0	38.46c
							1.0	27.03c
	V			1.0			1.0	27.5NS
							1.0	27.3NS
	VI			10			1.0	14.4d
							1.0	29.4d
	VII		0.1				1.0	41.06e
							1.0	29.3e
	VIII		1.0				1.0	36.3f
							1.0	28.4f
	IX				0.1		1.0	40.3g
							1.0	29.3g
	X				1.0		1.0	29.8NS
							1.0	32.6NS

[a] Test 1 was carried out in Cieza (Murcia, 1984) and test 2 in Mora de Rubielos (Teruel, 1985).
[b] Ten traps per treatment. Means within parcel followed by the same letter are significant at $P < 0.05$, Student's t test. NS = nonsignificant.
[c] Only two traps per formulation.

could not prepare the alternative positional isomer of ether **9**, 13-oxahexadec-11-ynyl acetate, due to its inherent chemical instability.

With regard to the variable effect of the formate **14**, it should be noted that, in other cases, blends of natural pheromones with other synthetic compounds can considerably increase or decrease trap catch, depending on the relative ratios used in the bait (Kamm and McDonough, 1980).

When propionate **15** was mixed with pityolure **1** in a 1:10 ratio (parcel

IX, test 2), an increase in the number of catches was observed, whereas no significant effect was found when both compounds were mixed in a 1 : 1 ratio (parcel X, test 2). This nonsignificant effect differs from the inhibitory action elicited by a mixture of the sex pheromone of the red-banded leaf roller moth and the corresponding propionate analog with the same relative ratio. Therefore, it might be assumed that effects shown by the same type of analogs are species-specific and that no general validity may be extrapolated from the results obtained in one particular case. On the other hand, when the allene **17** was tested in baits with pityolure **1** (1 : 1 ratio, parcel III, test 2), no appreciable variation in trap catches was observed.

A wide range of EAG activities was exhibited by compounds depicted in Scheme 1. While compounds **4**, **5**, *E*-**6**, *Z*-**7**, and *E*-**7** with very low EAG responses in comparison with that of pityolure **1** (<20%) were not further investigated, the intrinsic activity of the remaining analogs was studied in a new set of field experiments (Table 3). In comparison with the sex pheromone component, some of these analogs, such as epoxide **10**, ether **9**, (*E*,*Z*)-**8**, and acetylene **3**, had slight attractant activity. However, we found that acetylene **2** had a fairly good activity amounting up to 65% of the efficiency of natural compound **1** (Camps et al., 1987b). Likewise, propionate **15** was also a good pheromone mimic, with a 40% relative trapping efficiency.

Figure 1 shows the trap catch vs. time for the sex pheromone component **1**, propionate **15**, and acetylene **2**. Although the activity pattern of the propionate vs. pheromone is similar, the activity of acetylene **2** seems to be better than

TABLE 3. RELATIVE INTRINSIC ATTRACTANT ACTIVITY OF ANALOGS **2**, **3**, *Z*-**6**, **8**, **9**, **10**, **14**, AND **15** COMPARED WITH PITYOLURE **1** (Mora de Rubielos, 1985)

Attractant	Total catch[a]	Relative efficiency (%) vs. pityolure
9	123d	9
3	79d	6
2	858b	65
Z-**6**	9e	0.6
15	523c	40
14	8e	0.6
10	182d	14
1	1322a	100
Z,*Z*-**8**	5e	0.4
Z,*E*-**8**	18e	1.4
E,*Z*-**8**	106d	8
E,*E*-**8**	18e	1.4

[a] Ten replicates per trap. Values followed by the same letter are not significantly different at $P = 0.05$ (Duncan's multiple-range test).

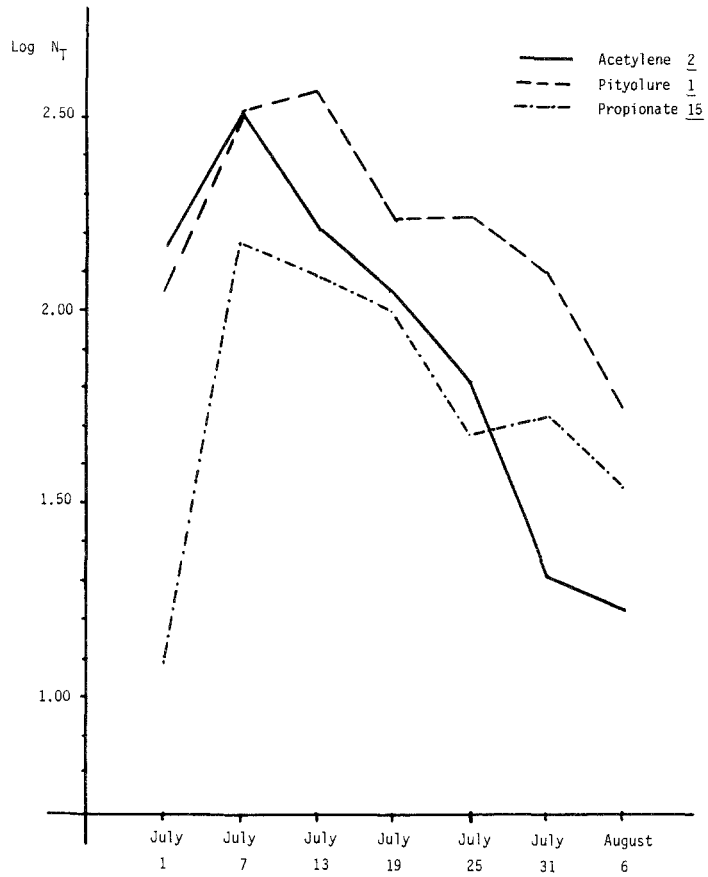


FIG. 1. Evolution of total number of catches of *Thaumetopoea pityocampa* males by lures baited with 1 mg of pityolure **1**, acetylene **2**, and propionate **15** (Mora de Rubielos, 1985).

propionate **15** except for the last part of the experiment. This was initially rationalized in terms of the possibly higher inhibitory effect of alcohol **13**, resulting from partial hydrolysis of the parent acetylene **2**, when compared with that of alcohol **12**, similarly originated from the natural pheromone **1** and propionate **15** under environmental conditions. However, analysis of the corresponding carriers after field trials showed a very small amount of alcohols **12** and **13**.

Furthermore, as shown in Table 4, in field tests designed to investigate the above hypothesis, it was found that the inhibitory effect shown by alcohol **12** on pityolure **1** was apparently higher than that promoted by alcohol **13** on its

TABLE 4. INHIBITORY EFFECTS OF ALCOHOLS **12** AND **13** AND TRIFLUOROACETATE **16** ON PITYOLURE **1** AND ACETYLENE **2** (Mora de Rubielos, 1986)

	Bait formulation (mg)					Total No.
Parcel[a]	Alcohol **12**	Alcohol **13**	Acetylene **2**	Trifluoroacetate **16**	Pityolure **1**	of catches[b]
I	0.1				1	72b
	1				1	82b
	10				1	64b
					1	540a
	1					10b
II		0.1			1	350abcd
		1			1	389abc
		10			1	414ab
					1	449a
		1				6e
III		0.1	1			220b
		1	1			138bc
		10	1			105bcd
			1			343a
		1				19d
IV				0.1	1	140b
				1	1	44c
				10	1	38c
					1	228a
				1		4c

[a] Several km of distance between parcels.
[b] Five replicates per trap. Means followed by the same letter are not significantly different at $P = 0.05$ Duncan's multiple-range test.

parent compound **2**. These results were further confirmed in EAG bioassays (see below). Other alternative possibilities to explain these differences in the activity pattern of these compounds are being considered in planning further studies. On the other hand, when alcohol **13** was mixed with pityolure **1**, no significant decrease in catches was observed. This fact combined with the clear inhibitory effects shown by alcohols **12** and **13** on pityolure **1** and the acetylene **2**, respectively, may suggest the presence of two different specific receptor cells, with high affinity for compounds **1** and **2**. However, this assumption has not been appropriately substantiated by cross-effect experiments or single-cell recording studies.

Remarkably, the trifluoroacetate **16**, although intrinsically inactive, inhibited significantly the activity of the sex pheromone component when mixed with **1** in 0.1 : 1, 1 : 1, and 10 : 1 ratios (Table 4). Both effects were also confirmed in EAG bioassays by presaturation of the antennal receptors for 4 hr with dif-

ferent amounts (0.1, 1, 10, 100, and 1000 μg) of trifluoroacetate **16** and further exposure to pheromone (10 μg) "puffs," whereby a very small response was recorded. In addition, we have found that, under field conditions, trifluoroacetate **16** is not substantially hydrolyzed to alcohol **12**, which might be held responsible for this inhibitory effect. In this context, Albans et al. (1984) observed an antipheromone action in field trials with the trifluoroacetate analog of the sex pheromone of a different species (*Heliothis virescens*).

Conclusions. Selected structural modifications of the three putative critical molecular parts of pityolure **1** have led to the synthesis of analogs **2–17**, which have exhibited diverse effects, mimicking, enhancing, or decreasing the activity of the sex pheromone component in field and laboratory bioassays. In general, preservation of the acetylene group at C-11 of the original structure is shown to be essential for high activity. On the other hand, fluorine substitution for vinyl hydrogen at C-13 or replacement of the acetate moiety by a trifluoroacetate group induced antipheromone activity. Likewise, alcohols **12** and **13** exhibited inhibitory effects on the corresponding acetates. In addition, synergistic effects have arisen from the transformation of the double bond into the corresponding epoxide or into an oxymethylene group, as well as when the acetate group was replaced by propionate or formate functions. However, surprisingly, in this latter analog, the synergistic action could be reversed into inhibition, according to the relative ratios used in the bait, when mixed with the sex pheromone component.

It is noteworthy to point out that the conjugated enyne moiety of the major component of the female sex pheromone of *Thaumetopoea pityocampa* has not been found in any other insect pheromone structure. From our results, it appears that, in this unique moiety, the triple bond is essential to elicit pheromonal activity.

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