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Sex Pheromone of the Oak Processionary Moth *Thaumetopoea processionea*. Identification and Biological Activity

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The sex pheromone of the oak processionary moth *Thaumetopoea processionea* has been characterized from female gland extracts as a mixture of (*Z,Z*)-11,13-hexadecadienyl acetate (**1**), (*E,Z*)-11,13-hexadecadienyl acetate (**3**) and (*Z,Z*)-11,13-hexadecadienol (**2**) in 88:7:5 ratio. The amount of the major compound **1** was 20−30 ng/gland. No trace of (*Z,Z*)-11,13-hexadecadienal was found in the extract, and therefore, *T. processionea* appears to be the only "summer" processionary moth lacking this compound as a pheromone compound. The alcohol **2** had also been previously found but is electrophysiologically inactive, and in wind tunnel assays it lowers the number of contacts with the source when mixed with the major compound **1**. The major component **1** elicited males to display the complete behavioral sequence, but the amount of chemical needed was unexpectedly high in comparison to the activity displayed by virgin females and gland extracts. (*E,E*)-11,13-hexadecadienyl acetate (**5**) inhibits the attractant activity of the major component **1** when mixed with **1** in 1:10 and 1:1 ratios. The main constituent **1** is active in the field, but its tendency to isomerize into the corresponding *E,E* isomer (**5**) must be considered if effective formulations are to be prepared.

KEYWORDS: ***Thaumetopoea processionea*; oak processionary moth; sex pheromone; pest control**

INTRODUCTION

Thaumetopoea processionea L. (Lepidoptera, Thaumetopeidae) is one of the most serious defoliators of oak trees in many areas of western, southern and central Europe. The affected trees resprout, but the wood production is slowed, and the vitality of the trees is weakened (*1*). Even more serious than the forest damage is the health hazard caused by these insects. As with other species of the genus *Thaumetopoea*, the caterpillars carry urticating hairs that can cause strong allergic reactions in humans and animals. Frequently, the skin (contact-dermatitis) and the eyes (conjunctivitis, keratitis, uveitis) are affected, and irritation of the respiratory mucous membranes may cause asthma and even anaphylactic shock (*2*, *3*). The oak processionary moth prefers open stands and well insulated trees, thus buildings and infested sites may lose some of their economic value. Cultural events are often canceled, and recreational parks and camping sites have to be closed (*4*). In such public areas, control of *T. processionea* by conventional methods is often difficult (*5*). Moreover, there is a lack of suitable methods to map and detect infestation areas sufficiently early.

A promising way of detecting and controlling the pest might be the use of the sex pheromone. Unfortunately, little is known about the sex pheromone of *T. processionea.* In Italy, Tiberi and Niccoli (*6*) initially mentioned that traps baited with *T. pityocampa* sex pheromone, (*Z*)-13-hexadecen-11-ynyl acetate, also captured males of *T. processionea*, but this was not confirmed later in field trials conducted in Belgium (*7*) and in Spain (Montoya and Hernández, personal communication). In 1993, Frérot and Démolin described (*Z,Z*)-11,13-hexadecadienyl acetate (**1**) and (*Z,Z*)-11,13-hexadecadienol (**2**) as possible pheromone components (*8*), but no data on their biological activity were disclosed. Also, Germinara et al. (*9*) found compound **1** in extracts of virgin female glands, but again no biological activity was available. To our knowledge, no other reports have been found in the literature (*10*). In the present study, the structure of the sex pheromone of the oak processionary moth was characterized by spectroscopic (GC-MS) and electroantennographic (EAG, GC-EAG) techniques and the biological activity of its components both in the laboratory and in the field was investigated.

Insects. *T. processionea* nests were collected in highly infested areas in Belgium just after pupation of the caterpillars. All work was done wearing protective clothing, and the nests were stored under a well-ventilated hood to provide protection from the highly urticating allergenic hairs. The cocoons of the

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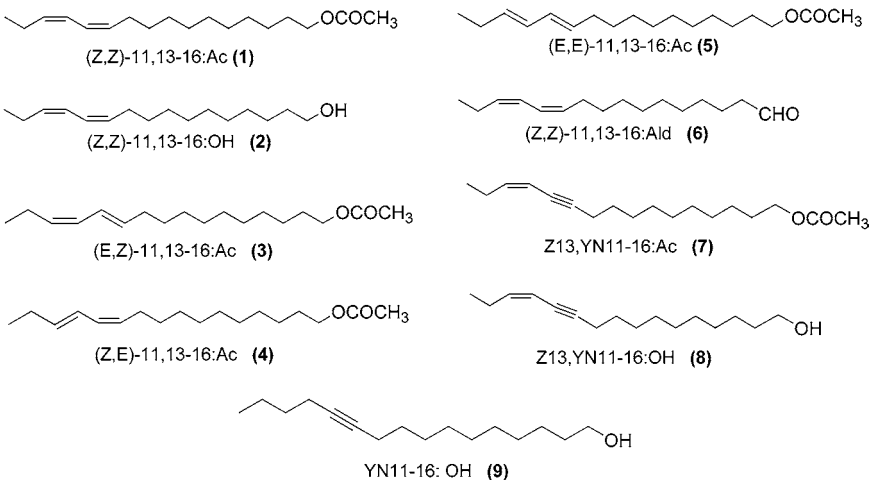

Figure 1. List of pheromone compounds and analogues considered.

nests were carefully opened, and the pupae were collected, sexed, washed, and sent to Barcelona for the characterization of the sex pheromone and electrophysiological and wind tunnel experiments. Upon reception, pupae were placed in plastic boxes (31 × 12 × 12 cm) and covered with a 1 cm layer of sawdust. They were kept in a light/dark (16:8) reversed photoperiod at 23 ± 1 °C and 55−65% humidity until eclosion. Toothpicks were placed standing in the sawdust to favor hatching of the moths and expanding their wings.

Chemicals. Compounds **1**−**9** were synthesized by conventional methods and kindly provided by Sociedad Española de Desarrollos Químicos (Barberá del Vallés, Barcelona, Spain) (**Figure 1**). The chemicals were purified in our laboratory by preparative high-performance liquid chromatography (HPLC) on a 25 cm × 1 cm i.d. × 5 μm Kromasil 100 C-18 column (Tracer Analítica, St. Cugat, Barcelona, Spain) eluted with a mixture of acetonitrile/water (9:1). Stereochemical analyses were done by GC on a 25 m × 0.20 mm i.d. × 0.33 μm cross-linked HP-FFAP capillary column (Agilent Technologies, Barcelona, Spain) and on a 12 m × 0.2 mm i.d. × 0.33 μm HP-1 column (Agilent Technologies, Barcelona, Spain).

Gland Extracts. Because virgin females usually call during the first scotophase after emergence, groups of four females were taken out of the storage room 4 h after reaching the scotophase and anesthetized with CO_2, and the glands were extruded manually. They were dissected, placed in a vial with 100 μL of hexane, and kept for 1 h at room temperature. The glands were removed, and the extracts were stored at −80 °C until use. For GC or GC-MS analyses, the solvent was concentrated to 2−3 μL, and the whole extract was immediately injected for analysis either on the cross-linked HP-FFAP capillary column or on a 30 m × 0.25 mm i.d. × 1.0 μM BPX-35 column (SGE, Ingeniería Analítica, St. Cugat, Barcelona, Spain). Solid-phase microextraction (SPME) analyses were carried out by rubbing a 100 μm poly(dimethylsiloxane) fiber (Teknokroma, St. Cugat, Barcelona, Spain) over the gland surface of 2 calling females for 30 s. The volatiles were analyzed on a 30 m × 0.25 μm i.d. × 1 μm BPX-5 column (Ingeniería Analítica, St. Cugat, Barcelona, Spain).

Electrophysiology. EAG responses were obtained in an EAG apparatus, previously set up in our laboratory (*11*) and provided with a signal interface box and a data acquisition interface board (Syntech, Hilversum, The Netherlands). Depolarization values were monitored in case of decrease in antennal sensitivity during the experiments by intermittent application (once every three stimulations of the test compounds) of puffs over a Pasteur pipet containing a filter paper with 1 μg of (*Z*,*Z*)-11,13-hexadecadienyl acetate (**1**), which was also used as reference stimulus. However, for all replicates ($n = 6$ insects, 1 antenna/insect) the response to the reference stimulus did not decay throughout the tests. All test stimulations within each replicate were normalized to the averaged reference responses. Puffs were made with 1 min intervals, and the order of presentation of the test stimuli was randomized among replicates. The normalized responses were plotted relative to the mean EAG value to compound **1**.

GC-EAG responses were obtained from a setup previously described by us (*12*). The GC system used a Vega series 6000 gas chromatograph (Carlo Erba, Rodano, Italy) equipped with a 25 m × 0.20 mm i.d. × 0.33 μm HP-FFAP fused silica capillary column (Agilent Technologies, Barcelona, Spain) with hydrogen as carrier gas and nitrogen as makeup gas. At the end of the capillary column, a variable outlet splitter (SGE, Ingeniería Analítica, St. Cugat, Barcelona, Spain) split the effluent to the flame ionization detector and to the transfer line to the EAG preparation. At the entrance of the valve, a second inlet of makeup gas (30 mL/min) was used to reduce residence time in the transfer lines and broadening of the peaks. The GC program used was the following: injection at 100 °C for 1 min, increased at 15 °C/min to 190 °C, then 8 °C/min to 225 °C, and held at this temperature for 20 min.

Wind Tunnel Assays. Tests were carried out in a glass wind tunnel (180 × 55 × 50 cm) provided with a video camera (Pulnix B/W TM50) (Sonimed, Barcelona, Spain) placed 135 cm above the tunnel in perpendicular position to minimize optical distortion of the flight. The camera was linked to a JVC SR306E video recorder and a Panasonic TC-14S1RC monitor. Illumination (2−4 lux) was provided by a dimmed fluorescent red light. Assays were conducted as previously described (*13*). When virgin females were used as attractants, one specimen was placed in a 6.5 × 4 × 3 cm stainless steel cage of 0.2 × 0.2 cm mesh. All types of behavior (wing fanning and taking flight, arrival to the middle of the tunnel, close approach to the lure and contact with the source) were recorded. Assays were conducted between the fourth and sixth hours of the first scotophase, and males were used only once. The wind speed was 22 cm/s, light intensity was 2−4 lux, temperature was 24−26 °C, and the relative humidity was 60−80%. Test chemicals were deposited as hexane solutions on Whatman filter paper squares (2 × 2 cm), which were hung 130 cm away from the release point of males in the far end of the wind tunnel. The tests were run to determine the activity of the major compound **1** and the presence of possible inhibitors in the extract.

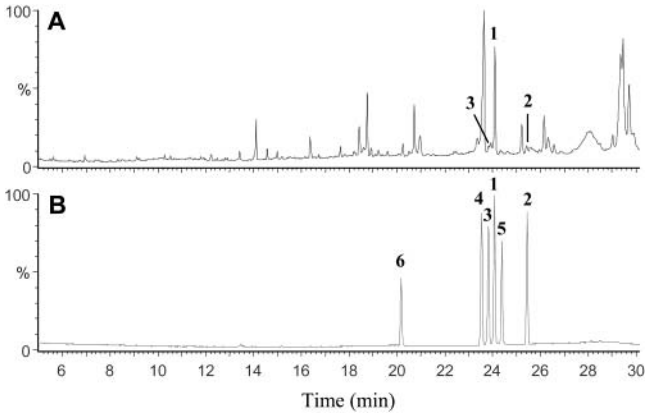

Figure 2. A. GC-MS analysis of an extract of four pheromone glands of *T. processionea*. B. GC profile of a mixture of synthetic compounds **1**–**6** as reference.

Field Tests. Field trials were carried out in an infested oak forest in Germany (Genthin, NE of Magdeburg) from July 27 to August 22, 2001. For the tests, two baits were prepared, containing 10 mg of the major component, either *Z*,*Z* or *E*,*E* isomer, and BHT (1%) as stabilizer. The formulations were encapsulated in 3 cm × 1.1 cm i.d. polyethylene vials (Sociedad Española de Desarrollos Químicos, Barberá del Vallés, Barcelona, Spain) and placed in Delta traps (Servei de Protecció dels Vegetals, Generalitat de Catalunya). Each treatment was replicated five times with traps hung on oak trees at a height of 10−15 m above the ground. Traps placed at lower height (2 m or 6−8 m) were clearly less efficient (unpublished results). The distance between adjacent traps was about 50 m. The traps were inspected at 2−4 d intervals and rotated to minimize effects of their location in the forest. The moths were counted, and the adhesive bottom was renewed when necessary.

RESULTS AND DISCUSSION

GC-MS analyses of a pool of a four-gland extract showed the presence of (*Z*,*Z*)-11,13-hexadecadienyl acetate (**1**) as the major compound (ca. 20−30 ng/gland) along with very small amounts of (*E*,*Z*)-11,13-hexadecadienyl acetate (**3**) and (*Z*,*Z*)-11,13-hexadecadienol (**2**) (**Figure 2**) in 88:7:5 ratio by comparison of their MS spectra with authentic synthetic standards. Also, the compounds displayed identical gas chromatographic behavior on a polar (HP-FFAP) column, which efficiently separated the complete set of stereomeric acetates *Z*,*Z* (**1**), *Z*,*E* (**4**), *E*,*Z* (**3**) and *E*,*E* (**5**) (retention times: **3**, 12.52 min; **1**, 13.01 min; **2**, 13.46 min; **4**, 12.43 min; **5**, 13.10 min). A large peak with a retention time similar to the *Z*,*E* isomer (**4**) also appeared in the gland, but further analysis on a BPX-35 column revealed that both chemicals were not identical. In addition, this large peak elicited no electrophysiological responses in GC-EAG analyses (see below). Our analyses confirm the presence of compounds **1** and **2** as components of the pheromone complex described by Frérot and Démolin (*8*) but also show isomer **3** as minor constituent. Interestingly, no trace of the corresponding aldehyde (*Z*,*Z*)-11,13-hexadecadienal (**6**) was found in the extract by comparison with an authentic sample. *T. processionea* appears to be the only "summer" processionary moth lacking this chemical as a pheromone component, because the other closely related species *T. pinivora* (Tr.) and *T. bonjeani* (Powell) both utilize **6** as their major pheromone constituent (*8*).

GC-EAG of an extract of one pheromone gland showed that compounds **1** and **3** were the only electrophysiologically active compounds, whereas alcohol **2** elicited no activity (**Figure 3**). When the antennal receptors were stimulated with the major compound **1**, analogues **2**−**9**, and mixtures thereof, compound **1** elicited the highest depolarization, similar to the ones displayed by mixtures of **1**/**4**/**5**/**3** in 77:12:5:6 ratio or **1**/**4**/**3** in 81:13:6 ratio (**Figure 4**). The acetate analogues **3**, **4**, and **5** showed significantly lower electrophysiological activity (35−50%) than **1**. Alcohol **2**, although present in the gland, was practically inactive (5.4% relative to compound **1**), which raises the question concerning its role in the gland. This alcohol may be a precursor of the major component that has not been transformed into the corresponding acetate or an artifact resulting from hydrolysis of the parent compound. In the pine processionary moth *T. pityocampa*, the alcohol precursor of the major and only pheromone component (*Z*)-13-hexadecen-11-ynyl acetate (**7**) (*14*) was undetected in gland extracts but proved to be a potent inhibitor of the attraction evoked by the major component **7**, both in the laboratory (*13*) and in the field (*15*). In the oak processionary moth, alcohol **2** also reduced the number of source contacts (SC) when mixed with 10 μg of the major compound **1** in 5:95 (19% SC, $N = 26$) and 10:90 ratios (8% SC, $N = 13$), relative to the activity of **1** alone (32% SC, $N = 34$), and when mixed with 100 μg of **1** in 10:90 ratio (17% SC, $N = 12$) in comparison to the effect of the same amount of **1** alone (40% SC, $N = 10$) (data not shown). The other analogues tested, including pheromone and analogues of the pine processionary moth, were electrophysiologically inactive (**Figure 4**).

In the wind tunnel, 68−80% of *T. processionea* males locked onto the plume when presented with 10 μg or higher amounts of the major isomer **1**. However, only 32−40% successfully flew upwind and contacted the source (**Figure 5**). This percentage was significantly lower than that elicited by one virgin female but was similar to a three-gland extract. However, the large quantity of the major isomer required to attract males and induce landing on the source in comparison to the amount of the compound present in the gland (20−30 ng) is surprising. Consequently, we hypothesized that the presence of small quantities of a possible inhibitor in the synthetic sample of **1** might account for this result. The possibility that there might be a missing compound that could be produced at the moment of release was considered by SPME analysis of volatiles. Only one compound appeared at a retention time of 16.38 min, close to the one corresponding to the *Z*,*Z* isomer (**1**, 16.26 min), whereas the retention times of the other isomers were 15.84 min for the *Z*,*E* isomer (**4**), 15.96 for the *E*,*Z* isomer (**3**), and 16.14 for the *E*,*E* isomer (**5**). No other compound was apparent in the extract. Wind tunnel studies were then directed to the search of a possible inhibitor. When mixtures of the *Z*,*Z* (stereochemical purity *Z*,*Z* 92.9%; *Z*,*E* 3.8%; *E*,*Z* 2.8%; *E*,*E* 0.2%) and the *E*,*E* isomer (*Z*,*E* 13.1%; *E*,*Z* 11.1%; *Z*,*Z* 2.9%; *E*,*E* 72.8%) in 100:1, 10:1, and 1:1 ratios were tried as lures, the number of males contacting the source was lower than with the *Z*,*Z* isomer alone, the difference being significant at 10:1 and 1:1 ratios (χ^2 homogeneity test, $P < 0.05$). Thus, whereas 100 μg of **1** attracted 27 males out of 73 (37%) to contact the source, the 100:1 *Z*,*Z*/*E*,*E* mixture lured 3 out of 20 (15% SC), the 10:1 mixture lured 8 out of 57 (14% SC), and the 1:1 mixture attracted only 5 out of 58 (9% SC). Therefore, the *E*,*E* isomer appears to reduce the attractant activity of the major component in the wind tunnel when mixed with this chemical in 1:10 or higher ratios.

The activity of the major component **1** was also evaluated in the field. Delta traps baited with 10 mg of the chemical (stereochemical purity *Z*,*Z* 93.7%; *Z*,*E* 3.9%; *E*,*Z* 2.2%; *E*,*E*

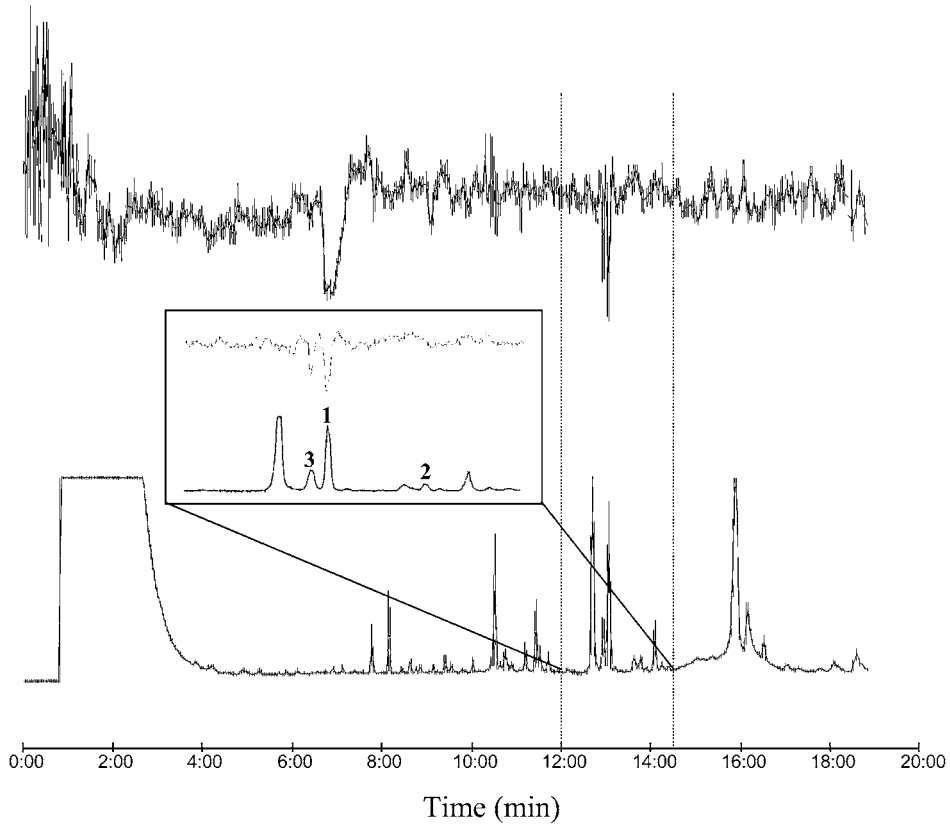

Figure 3. GC-EAG trace of an extract of one pheromone gland of *T. processionea* using an HP-FFAP capillary column. Insert: amplified zone containing the active compounds. The depolarization in the EAG trace around 6–7 min is an artifact due to instability of the antenna.

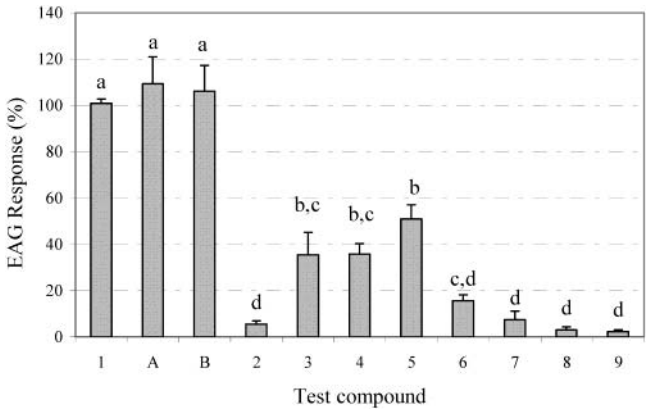

Figure 4. EAG relative response of *T. processionea* males antennae to 1 μg of (*Z,Z*)-11,13-hexadecadienyl acetate (**1**), several analogues (**2**−**9**), and mixtures thereof. A: mixture of analogues **1/4/5/3** in 77:12:5:6 ratio. B: mixture of analogues **1/4/3** in 81:13:6 ratio. Bars (± SE) with different letters represent significantly different values (ANOVA, LSD test, $P < 0.05$).

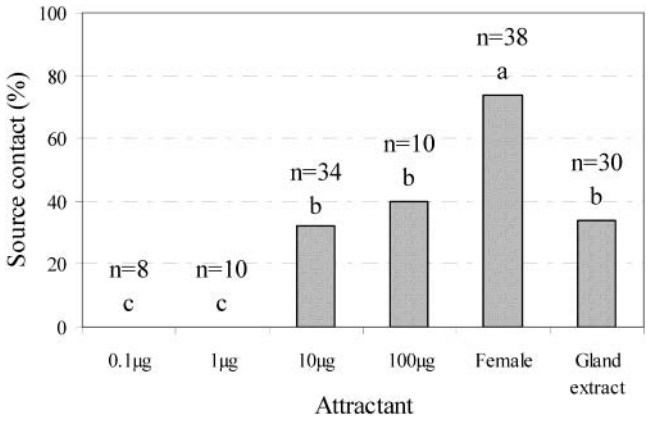

Figure 5. Source contact behavior of *T. processionea* males in response to different doses of (*Z,Z*)-11,13-hexadecadienyl acetate (**1**), the major component of the pheromone, in comparison to one virgin female and a three-gland extract. Bars with different letters represent significantly different values (χ^2 homogeneity test, $P < 0.05$) (n = number of insects tested).

0.1%) caught a mean number of 62.2 males/trap during the season, whereas a synthetic sample enriched with the *E,E* isomer (stereomeric purity *Z,E* 13.1%; *E,Z* 11.1%; *Z,Z* 2.9%; *E,E* 72.8%) attracted, on average, only 16.0 males/trap. These data confirm the attractant activity of the major constituent **1** and the very low activity, if any, of the *E,E* isomer, because the low 2.9% of the *Z,Z* isomer present in the bait may well account for the number of males caught.

In summary, the sex pheromone of the oak processionary moth has been characterized as mixture of (*Z,Z*)-11,13-hexadecadienyl acetate (**1**), (*E,Z*)-11,13-hexadecadienyl acetate (**3**) and (*Z,Z*)-11,13-hexadecadienol (**2**) in 88:7:5 ratio, although the alcohol is electrophysiologically inactive. In a wind tunnel, the major component evoked males to display all behavioral responses, but the amount of chemical needed was surprisingly high in comparison to the activity displayed by virgin females or gland extracts. The *E,E* isomer **5** appeared to reduce the number of contacts with the source when mixed with the major component in several ratios. In the field, the major constituent is active, but its tendency to isomerize into the corresponding *E,E* isomer should be taken into account for the development of efficient formulations to control the pest.

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