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IDENTIFICATION OF THE SEX PHEROMONE OF THE FURNITURE CARPET BEETLE, ANTHRENUS FLAVIPES LECONTE

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Virgin female furniture carpet beetles, Anthrenus flavipes, LeConte release a chemical that attracts males and stimulates sexual responses. The female emits this substance early in the photophase while she is in a stationary "headstand" posture; this activity is referred to as calling behavior[1]. The females are apparently signaling the males and enticing them to mate. Chemical tests on the female extract indicated that there is an extractable substance which elicits a normal sexual response in the males.

In an attempt to isolate this substance, we started from two lots of virgin females (Experiment A, 600; B, 4,300 females). They had been sexed individually and segregated at their pupal stage by determining their morphological secondary sexual characteristics with the aid of a dissecting microscope. In order to obtain as much pheromone as possible, the females were held on filter papers for up to 10 days after emergence and during this period they performed daily calling behavior. Both the females and the filter material were extracted with n-hexane.

The isolation procedure following the initial extraction was as follows: the attractant was eluted from a silicic acid column with chloroform and purified on a preparative silica-gel thin-layer chromatographic system with chloroform as the mobile phase. The active zone of the chromatogram was collected and the pheromone was eluted by using acetone. The eluate

was treated with diazomethane to form methyl esters which were injected to a gas-chromatographic system involving a 180 cm glass column of SE52 at 90°C. The active fraction was collected (peak at 11.6 min. under the experimental conditions as compared to 11.9 min. for a reference compound, methyl decanoate). From two independent trials, 15 μg (Experiment A) and 200 μg (Experiment B) respectively, of pure pheromone were obtained. The purity of each preparation was examined on the same gas-chromatographic system by using first a neopentylglycol column (NGA, 180 cm glass at 90°C) and then a carbowax 4000 column (180 cm at 70°C). In each case, the pheromonal activity was found to be aligned with a single symmetrical peak (retention times of 10.5 min. for the former and 11.0 min. for the latter system, as compared to methyl decanoate of 8.7 min. for both systems).

Throughout the study, the pheromonal activity was monitored by means of one-dram shell vial olfactometers[2] in the form of the original acid. However, during isolation attempts only, some of the samples were assayed in the form of the methyl ester to facilitate prompt identification of the active fractions since the methyl ester of the pheromone was also active as in the case of another dermestid beetle, _Trogoderma inclusum_ LeConte[3]. The derived pure methyl ester of the pheromone was hydrogenated by the method of Schwartz, _et. al._[4] to study its basic carbon structure. It was found that the hydrogenation product was identical to methyl decanoate as judged by their retention times on SE52 and NGA columns. The mass spectrum of the methyl ester of the pheromone was then taken by using an SE30 capillary (15 m, at 79.5°C) column coupled with a Finnigan 1015 GC-Mass spectrometer system. The parent mass (m/e) was found to be 184 corresponding to a methyl decenoate. Other major peaks were 152 (M-32), 123 (M-61) and 110 (M-74), the base peak being 73. The first two peaks indicate that this compound is a methyl ester of carboxylic acid as expected. To determine the site of unsaturation, the methyl ester of the original pheromone was first reduced by $LiAlH_4$ in ether to the corresponding alcohol and then esterified by using pentafluoropropionic anhydride[5]. The resulting compound was used as a marker by using a Varian gas-chromatographic system with an electron capture detector. The result of ozonolysis experiments (in pentane for 20 min. at 0°C) clearly showed the

position of the double bond to be 3, the ozonolysis product being identical to $OHC(CH_2)_2O$-(O)CC_2F_5 in 2 different gas chromatographic systems.

Under the circumstances there were only two possibilities remaining: the identity of the pheromone was either (E)-3 or (Z)-3-decenoic acid. Both compounds were synthesized[6]. Upon bioassay, it became clear that the (Z) isomer had 20 times as much biological activity as the (E) isomer, the threshold levels being 0.25 ng and 5 ng/disc/assay for (Z) and (E) isomers, respectively.

In order to demonstrate the similar biological activity of the synthetic (Z) isomer and the pure natural pheromone, two dilution series with concentrations of 0.05, 0.025, 0.01 and 0.005 µg were prepared for both samples. Each preparation was assayed with 50 unmated males. The response of the males to the two compounds was similar (Table 1). The non-threshold

Table I. Number of Males Responding to Anthrenus flavipes Pheromone (out of 50 males individually tested).

Concentration (µg) per filter paper disc	Synthetic (Z)-3-decanoic acid	Natural pheromone
0.050	42	43
0.025	32	31
0.010	25	24
0.005	11	10

quantal response analysis technique developed by Puri and Senturia[7] indicated, for example, that the estimated ED_{50} concentration of the natural pheromone was 0.0142 µg. The same estimate for the synthetic pheromone was ED_{50} = 0.0131. In both cases the estimated standard error of these estimates was 0.0033. Thus within statistical error the two substances elicited the same response behavior in fifty percent of the insects. The chemical identities of the natural pheromone and the synthetic (Z)-3-decenoic acid were also critically established by preparing pentafluoropropionates from corresponding alcohols and by examining their retention times on GLC systems with SE52, SE30, NGA, and SE30 (15 m capillary columns) by using an electron capture detector. The two compounds were

identical in behavior in all GLC tests. The 3-decenoic acids have been reported to exist in at least two biological sources: in Octopus dolfleini[8] and in a Lactobacceriacae sp.[9].

This is the first sex pheromone identified from the beetle genus, Anthrenus. Anthrenus flavipes was first established in the United States in 1911 on furniture stuffed with horsehair imported from Russia, and since has caused widespread damage to materials such as wool, hair, fur, feathers, bristles, horn, tortoise shell, silk, and to cellulose material stained with animal excreta[10]. The detection of this elusive and cosmopolitan insect by means of traps baited with synthetic pheromone could aid in pest management.

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