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PHEROMONES, 71[1]. IDENTIFICATION AND SYNTHESIS OF FEMALE SEX PHEROMONE OF ERI-SILKWORM, *Samia cynthia ricini* (LEPIDOPTERA: SATURNIIDAE)

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Summary: (4*E*,6*E*,11*Z*)-4,6,11-Hexadecatrienal and (4*E*,6*E*,11*Z*)-4,6,11-hexadecatrienyl acetate were identified as the major components of the sex pheromone of the eri-silkworm, *Samia cynthia ricini*, females.

The eri-silkworm moth *Samia cynthia ricini* Donovan (Lepidoptera: Saturniidae) is indigenous to China and Southeast Asia. It is an insect often used in electrophysiological investigations because of easy rearing, availability, and the size of its antennae. In the procedures used for isolation of insect brain hormones, it serves as an excellent monitor of hormonal activities[2]. Females of the eri-silkworm moth emit a sex pheromone which attracts conspecific males. A preliminary study, based on functional group tests followed by a bioassay, by Tomida and Ishii[3] has indicated that the major component of the pheromone is a diunsaturated conjugated aldehyde. We report here the complete identification and synthesis of the major components of the pheromone.

The intersegmental membrane between the abdominal segments 8 and 9[4] was excised from laboratory-reared, 3- to 5-day-old virgin females[5], during the period of maximum calling. The excised membranes were extracted with hexane (10 µl) and the extracts were analyzed by GC and GCMS. In the chromatogram (Fig. 1), two pheromone-like peaks were recognized by their mass spectra (Table 1). The electron-impact mass spectrum of the later-eluting component **2** showed that the molecular ion was *m/z* 278 ($C_{18}H_{30}O_2$). The signal at *m/z* 218 (arising from a loss of CH_3COOH from the molecular ion), the prominent ion *m/z* 43 (CH_3CO), and a small but significant ion at *m/z* 61 (CH_3COOH_2), indicated that the component **2** was a hexadecatrienyl acetate.

Table 1. Mass spectra [*m/z* and rel. intensity (%)] of pheromone components **1** and **2**, dimethylhydrazone **3**, and TCNE-adduct **4**

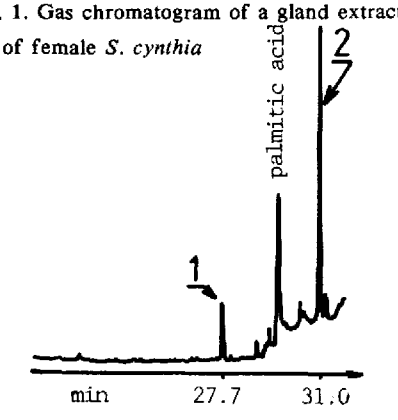
1 234(M^+, 3) 190(5) 150(18) 145(8) 134(25) 121(24) 119(29) 107 (11) 95(38) 93(38) 91(36) 82(43) 81(55) 80(47) 79(91) 67(90) 55(84) 41(100).

2 278(M^+, 12) 218(M^+-CH_3COOH, 7) 161(28) 150(13) 147(18) 137(9) 133(14) 121(21) 120(25) 119(32) 107(23) 105(41) 95(21) 94(24) 93(41) 92(18) 91(51) 81(49) 80(56) 79(100) 67(62) 61(3) 55(51) 43(91) 41(57).

3 276(M^+, 8) 86(6) 85(100) 44(21).

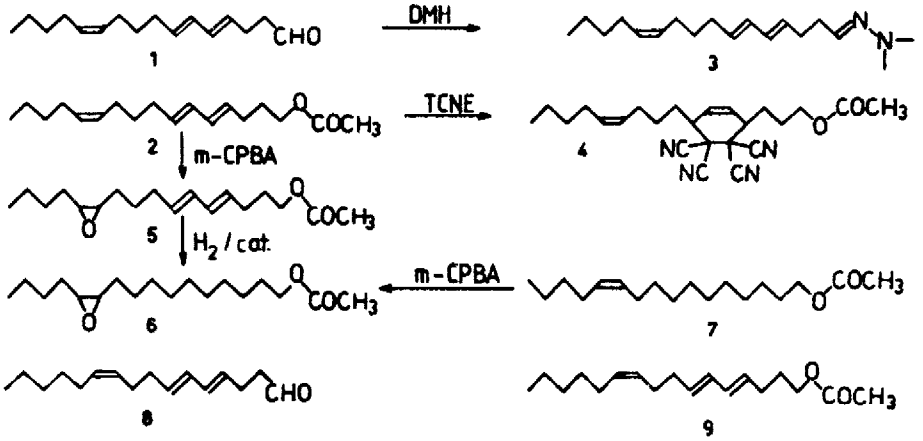
4 305(3) 61(13) 55(26) 43(100) 41(34).

Fig. 1. Gas chromatogram of a gland extract of female *S. cynthia*



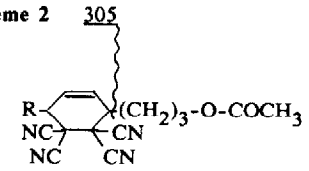
The presence of an aldehyde function in component 1 was confirmed by condensation with 1,1-dimethylhydrazine (DMH)[6] (Scheme 1). The biological activity of the extract, monitored as electroantennographic (EAG) responses, was drastically reduced after this reaction. The GC peak due to component 1 disappeared completely after the reaction and a new peak corresponding to hydrazone 3 appeared later in the chromatogram. The molecular ion of the mass spectrum of hydrazone 3 (Table 1) at *m/z* 276 corresponded to that of a triunsaturated hydrazone. The spectra of *N,N*-dimethylhydrazones of long-chain saturated aldehydes show a significant fragment, which is the base peak in most cases, at *m/z* 86, due to the formation of CH_2=CH-NH-$NMe_2]^{\ddagger}$ by a McLafferty rearrangement[7]. This ion is also the base peak of the spectra of unsaturated aldehydes, if the first double bond is not in the vicinity of the aldehyde group[7].The hydrazones of α,ß-unsaturated aldehydes are unable to undergo a McLafferty rearrangement; they experience an allylic fission to yield CH_2=CH-CH=CH-N=N^+Me_2 at *m/z* 111. The absence of an ion at *m/z* 111 in the spectrum of aldehyde-1-hydrazone 3 indicated that 1 is not an α,ß-unsaturated compound. A McLafferty rearrangement is also not possible in unsaturated aldehydes, when a double bond is present at C-4. The mass spectra of hydrazones of such unsaturated aldehydes yield a significant ion at *m/z* 85 due to the ion CH_2=CHN=N^+Me_2[8]. The presence of the ion *m/z* 85 as the base peak of the spectrum of 3 indicated that the first double bond is present at C-4. A mass spectrum obtained from the DMH derivative of 4,6,10-hexadecatrienal 8[9] was very similar to that of 3.

Scheme 1



The Diels-Alder addition of tetracyanoethylene (TCNE) to dienes is known to proceed stereospecifically, and at least for the conjugated dienes of R-CH=CH-CH=CH-R' type, only the (*E,E*)-isomers react with TCNE under mild conditions[10]. In fact, component 2 underwent reaction with TCNE to yield the adduct 4 (Scheme 1), confirming an (*E,E*)-conjugated diene system is present in 2. The mass spectrum of adduct 4 yielded a small, but significant and structurally diagnostic fragment at *m/z* 305 due to the loss of the alkyl side chain containing the acetate moiety (Scheme 2).

Scheme 2



4, 10

4 R = -$(CH_2)_3$C=C$(CH_2)_3CH_3$ (H H)

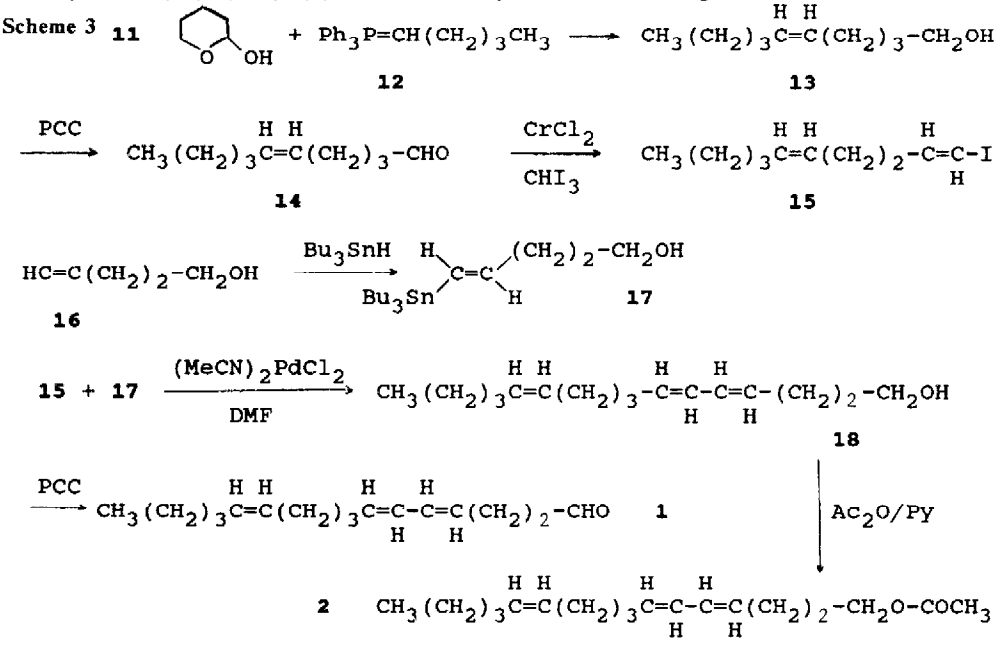
10 R = -$(CH_2)_2$C=C$(CH_2)_4CH_3$ (H H)

The mass spectrum of the TCNE-adduct **10** obtained from (4*E*,6*E*,10*Z*)-4,6,10-hexadecatrienyl acetate **9** was very similar to that of adduct **4** and also contained the ion at *m/z* 305. These results indicated the presence of a (4*E*,6*E*)-diene moiety in component **2**.

In order to solve the problem of the location of the third double bond in component **2**, we proceeded as illustrated in scheme 1: Acetate **2** was monoepoxidized by m-chloroperbenzoic acid (mCPBA) to **5**. The product **5** was hydrogenated by reaction gas chromatography[11] to **6**. The retention time of the hydrogenated product **6** on a polar SP-2340 capillary column was compared with those obtained from a series of positional and geometric isomers of epoxyhexadecyl acetates. The retention time of product **6** was identical to that of the epoxide obtained by the reaction of mCPBA with (*Z*)-11-hexadecenyl acetate **7**. These results allowed us to propose the structure (4*E*,6*E*,11*Z*)-4,6,11-hexadecatrienyl acetate for component **2**. Assuming a biogenetic relationship between component **1** and **2**, the component **1** was tentatively identified as (4*E*,6*E*,11*Z*)-4,6,11-hexadecatrienal.

In order to confirm the proposed identifications, the aldehyde **1** and acetate **2** were synthesized by a stereospecific cross-coupling reaction of vinyl halides with vinyl stannanes, catalyzed by palladium[12] as shown in scheme 3. 2-Hydroxytetrahydropyran **11** was reacted (*Z*)-selectively with pentylidene triphenylphosphorane **12** (silazide technique)[13] by Wittig reaction to give (*Z*)-5-decen-1-ol **13**. The alcohol **13** was subsequently oxidized with pyridinium chlorochromate (PCC) to (*Z*)-5-decenal **14**. (1*E*,6*Z*)-1,6-Undecadienyl iodide **15**, one of the two coupling synthons, was obtained by the reaction of **14** with $CHI_3/CrCl_2$[14]. The second coupling reagent, (*E*)-5-(tributylstannyl)-4-penten-1-ol **17**, was obtained by hydrostannylation of 4-pentyn-1-ol **16** according to ref.[15]. Cross-coupling of iodoalkadiene **15** with stannyl compound **17** using bis(acetonitril)dichloropalladium(II) $(MeCN)_2PdCl_2$ as catalyst[12] in DMF at room temperature yielded (4*E*,6*E*,11*Z*)-4,6,11-hexadecatrien-1-ol **18**, which was oxidized either to (4*E*,6*E*,11*Z*)-4,6,11-hexadecatrienal **1** or acetylated to (4*E*,6*E*,11*Z*)-4,6,11-hexadecatrienyl acetate **2** according to scheme 3.

Scheme 3



The mass spectra and GC retention times of synthetic 1 and 2 were identical to those obtained from natural 1 and 2. The synthetic aldehyde 1 elicited very high EAG activity whereas that of synthetic acetate 2 was moderate.

Preliminary behavioral tests show that a mixture of synthetic 1 and 2 releases wing flutter responses in males similar to those evoked by a gland extract. A sensory cell responding specifically to aldehyde 1 was found in male antennae by electrophysiological investigations (electrosensillogram, ESG). However, we have not yet been able to locate a cell stimulated by the acetate 2.

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