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Identification and Synthesis of the Sex Pheromone of *Phtheochroa cranaodes* (Lepidoptera: Tortricidae)

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Abstract: The pheromone of *Phtheochroa cranaodes* (Lepidoptera: Tortricidae) was identified as (3*E*,5*Z*)-3,5-dodecadienyl acetate (5) by GC-MS analysis of female gland extracts and field trapping of males with synthetic compound.

The leafroller moth *Phtheochroa cranaodes* Meyrick (Lepidoptera: Tortricidae) is, economically, the most important insect pest on apples in Southern Brazil. There is a need for a way to monitor this pest so that the farmers can know whether and when to use pesticides. Laboratory and field tests have shown that the female moths emit a sex pheromone which attracts conspecific males before mating.[1] Our analytical and electrophysiological study indicated that the pheromone was a dodecadienyl acetate. This female sex pheromone of *Phtheochroa cranaodes* was then identified as the 3*E*,5*Z*-isomer. Field tests indicate that traps baited with this pheromone can be used to monitor the pest.

RESULTS AND DISCUSSION

The leafrollers were reared in Brazil,[2] and sent to Sweden as pupae. Sex pheromone glands of calling females were extracted with heptane. Analysis of the extracts by GC-MS showed the presence of a number of acetates and methyl esters (Table 1). Among these, the dodecadienyl acetate, was tentatively identified as the

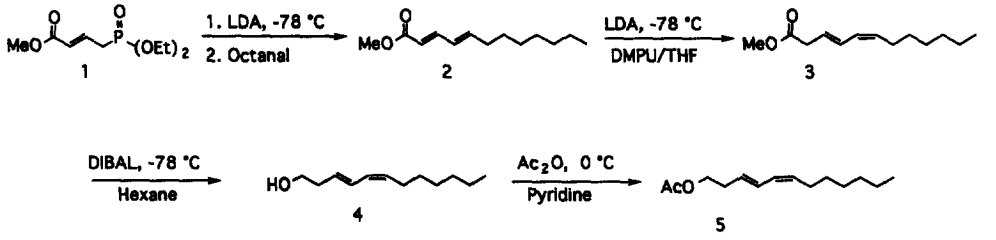
Table 1. Volatile Compounds Identified in the Extract of Female Abdominal Glands.

Compounds	MW	Relative amounts	Compounds	MW	Relative amounts
Decyl acetate	200	< 1%	Hexadecyl acetate	284	< 1%
Dodecyl acetate	228	< 1%	9Z-Hexadecenyl acetate	282	1-10%
3*E*,5*Z*-Dodecadienyl acetate	224	1-10%	Methyl octadecanoate	298	>10%
Tetradecyl acetate	256	< 1%	Methyl octadecenoate	296	1-10%
Methyl hexadecanoate	270	1-10%	Methyl octadecadienoate	294	>10%
Methyl hexadecenoate	268	1-10%	Octadecyl acetate	312	>10%

pheromone by electroantennographic detection (EAD).[3] The other gland compounds did not elicit a response in conspecific male antennae.

The mass spectrum of the dodecadienyl acetate was compared with the mass spectra of 4,11- and 5,11-dodecadienyl acetates and with the mass spectra of a number of conjugated dodecadienyl acetates published by Ando et al.[4] In comparison, the fragments 79, 80 and 164 (M-60) of our acetate gave very high peaks, and the molecular ion 224 a very weak one (<1%). The absence of an M+ peak indicated that the loss of the acetate group was somehow facilitated. If the unknown acetate was homoallylic, the intermediate radical formed after an initial McLafferty reaction would be allylic and therefore easily formed. The fragment 164 would then be formed after a charge migration and a subsequent alpha cleavage. The strong characteristic peaks 79 and 80 suggested the highly unsaturated molecular ions C_6H_7 and C_6H_8 which were possibly formed by allylic alpha cleavage and loss of acetic acid from 2,4-dodecadienyl acetate. It seemed likely, then, that the unknown acetate was homoallylic or allylic.

With this in mind we reduced a commercial mixture of ethyl decadienoates (International Flavors and Fragrances, IFF) with diisobutylaluminium hydride (DIBAL) and the alcohols formed were subsequently acetylated. This procedure resulted in a mixture of 2,4- and 3,5-decadienyl acetates. The mass spectra of the 2,4-isomers exhibited high M+ ion peaks, while the mass spectra of the 3,5-isomers were very similar to the one of our unknown acetate. Therefore, the synthesis of 3,5-dodecadienyl acetate was undertaken.



Scheme 1.

Synthesis of 3E,5Z-dodecadienyl acetate. A Wadsworth-Horner-Emmons reaction was used to prepare two methyl 2,4-dodecadienoates from methyl 4-diethylphosphonate-crotonate (1) and octanal (Scheme 1) according to literature procedures.[5] A strong base of low nucleophilicity at low temperature was used in order to deconjugate the 2,4-dodecadienoates to a mixture of 3*E*,5*Z*- and 3*E*,5*E*-dodecadienoates, the 3*E*,5*Z*-isomer dominating.[6] The 3,5-dodecadienoates were reduced with DIBAL and the alcohols formed were subsequently acetylated. The 3*E*,5*Z*-dodecadienyl acetate was separated from isomeric acetates by liquid chromatography on silver-impregnated silica gel.

The retention time and the mass spectrum of 3*E*,5*Z*-dodecadienyl acetate were the same as those of the biologically active compound. In order to confirm the assignment of the stereochemistry, a sample of 3*E*,5*Z*- and 3*E*,5*E*-dodecadienyl acetate was isomerized to all four geometrical isomers by use of UV-light (Rayonet photoreactor). This isomer mixture was then coinjected with the gland extract on two GC columns with different polarities. The unknown compound was co-eluted with 3*E*,5*Z*-dodecadienyl acetate from both columns.

3*E*,5*Z*-dodecadienyl acetate has earlier been found to attract *Chionodes lugubrella* in a field screening experiment,[7] but it has not been identified as a lepidopteran pheromone.

Behaviour tests. Wind tunnel experiments revealed that males of *Phtheochroa cranaodes* were attracted to synthetic 3*E*,5*Z*-dodecadienyl acetate. Furthermore, the males showed the same wingfanning response as males exposed to the female gland extract. Field tests were done in apple orchards in Vacaria, Brazil. Three trap baits, pure (>99 %) 3*E*,5*Z*-diene, a mixture of 40% 3*E*,5*Z*- + 60% 3*E*,5*E*-dodecadienyl acetates and hexane as control, were formulated at 100 µg on red rubber septa (ABS, Dietikon, Switzerland).[8] The field tests showed that pure 3*E*,5*Z*-dodecadienyl acetate attracted as many males as traps baited with live females, 1264 and 1317, respectively, and twice as many males as the isomeric blend (which attracted 603 males).

EXPERIMENTAL

Analyses and identifications. Sex pheromone glands of calling females were extracted in heptane (Merck pa)[9] and the extracts were analysed using two Finnigan SSQ 7000 GC-MS instruments connected with Varian 3400 GCs. Injections were made in a splitless mode for 30 seconds at 200 °C using helium as the carrier gas. One instrument was equipped with a DB-5MS fused silica capillary column (J&W 30 m, id 0.25 mm, film thickness 0.25 µm) programmed from 70 °C (hold 1 min) at 6 °C/min to 140 °C and at 3 °C/min to 200 °C. The retention times of the *E,Z, Z,E, Z,Z* and *E,E* isomers were 20:48; 20:53; 21:35 and 21:55 min, respectively. The other instrument was equipped with a DB-WAX column (J&W 30 m, id 0.25 mm, film thickness 0.25 µm) and used with a temperature programme of 70 °C (1 min) 6 °C/min to 140 °C followed by 2 °C/min to 200 °C. Coinjections of the gland extract with the mixture of the four 3,5-isomers were made on both columns.

Liquid chromatography (LC) was performed on silica gel (Merck 60, 0.040-0.063 mm) in 15 or 25 mm inner diameter glass columns with gradient elution, using hexane and increasing amounts of ethyl acetate.[10] NMR spectra were recorded in $CDCl_3$ on a Bruker AM 400 spectrometer. Analytical GC was performed with an FID detector using a 30 m DB-FFAP fused silica capillary column. Chemicals were obtained from Lancaster Synthesis Ltd if not otherwise stated.

Methyl 2,4-dodecadienoates. Lithium diisopropylamine was prepared by adding butyllithium (2.5 M, 52.6 ml, 131 mmol) to diisopropylamine (13.3 g, 131 mmol) in 180 ml dry THF at < 0 °C. The reaction mixture was cooled to -78 °C and methyl 4-diethylphosphonate-crotonate (1) (25.8 g, 124 mmol) in dry THF (40 ml) was added. The mixture was stirred for 1 h after completed addition and then octanal (16.5 g, 129 mmol) was added. The cold bath was removed and the reaction mixture was stirred for 2 h before it was poured into sat. NH_4Cl solution (100 ml). The organic phase was separated and the aqueous phase extracted with hexane (200 ml) and diethyl ether (2x150 ml). The combined organic phases were then dried with $MgSO_4$. After filtration the product was subjected to LC. The yield was 56 % (14.5 g, 69.0 mmol) of an isomeric mixture consisting of (*E,Z*):(*E,E*) in the ratio 20:80 according to GC. ^{1}H NMR δ: (**2*E*,4*E***) 7.29-7.23(m, 1H), 6.16-6.12(m, 2H), 5.78(d, J=15.4Hz, 1H) 3.73(s, 3H), 2.16(q, J=6.7Hz, 2H), 1.42(m, 2H), 1.28(m, 8H), 0.88(t, J=6.9Hz, 3H). ^{13}C NMR δ: (**2*E*,4*E***) 167.7, 145.4, 145.0, 128.3, 118.6, 51.4, 33.0, 31.8, 29.12, 29.07, 28.7, 22.6, 14.1. MS *m/z* (relative intensity); (**2*E*,4*E***): 210(M, 35), 111(100), 81(68), 93(53), 82(44), 79(40), 67(34), 179(25), 136(24), 100(22) (**2*E*,4*Z***): 210(M,10), 111(100), 81(18), 79(17), 67(11), 113(11), 100(9), 179(8), 139(6).

Methyl 3,5-dodecadienoates. A solution of lithium diisopropylamine (70 mmol) in dry THF (60 ml) was prepared as above and then the two methyl 2,4-dodecadienoates (13.5 g, 64.3 mmol) in DMPU (*N,N'*-dimethylpropylenurea) (60 ml) were added dropwise (during 1 h) at -70 °C. After stirring for 1 h the reaction was quenched by pouring the cold reaction mixture on an ice/sat. NH_4Cl solution mixture (150 ml). The reaction mixture was extracted with hexane (4x125 ml). After drying ($MgSO_4$), filtration and concentration, the residue was subjected to silica gel LC. The yield was 9.94 g of product consisting of (3*E*,5*Z*):(3*E*,5*E*):(2*E*,4*E*) in the ratio 54:25:21 according to GC. We suspect that the peak of the 2*E*,4*Z*-isomer (≈3%) is hidden in the 3*E*,5*Z*-peak.

2,4- and 3,5-Dodecadien-1-ols. A fraction of the mixture of methyl dodecadienoates (7.47 g, 35.6 mmol) obtained in the previous step was dissolved in dry hexane (300 ml) and DIBAL (13.3 ml, 10.6 g, 74.7 mmol) was added at 0 °C. After stirring for 3.5 h the reaction was quenched with the Baeckström reagent (14 g of celite/$NaSO_4$, 1:1 by vol.).[10] The reaction mixture was filtered and washed with aq. NH_4Cl and water. The aqueous phases were extracted with hexane and diethyl ether. The combined organic phases were dried ($MgSO_4$) and concentrated before they were subjected to LC. The yield was 5.57 g (86 %) and the isomeric ratios (3*E*,5*Z*):(2*E*,4*Z*):(3*E*,5*E*):(2*E*,4*E*) of the products were 51:3:23:23 according to GC. This mixture was used as such in the next reaction step. MS *m/z* (relative intensity) (**3*E*,5*Z***): 182 (M, 30) 67(100), 79(52), 81(44), 95(33), 41(32), 109(18), 151(3) (**3*E*,5*E***): 182 (M, 45), 67(100), 81(50), 79(42), 95(54), 43(22), 109(22), 151(4) (**2*E*,4*E***): 182 (M, 13), 83(100), 84(95), 41(68), 79(45), 54(36), 110(10), 164(9).

(3*E*,5*Z*)-3,5-Dodecadienyl acetate (5). The mixture of 2,4- and 3,5-dodecadienols (3.03 g, 16.7 mmol) was stirred in acetic anhydride and pyridine (10 ml, 1:2 v/v) at 0 °C for 1 h and at room temperature for 2 h. The reaction mixture was washed with water (2x50 ml), the aqueous phase extracted with diethyl ether and the combined organic phases were dried ($MgSO_4$). The reaction product was chromatographed, firstly on silica gel yielding 2.2 g of acetates (59 % yield) and then on $AgNO_3$-impregnated silica gel (15% w/w). A fraction consisting of 99 % pure 3*E*,5*Z*-isomer was isolated (133 mg). ^1H NMR δ: (**3*E*,5*Z***) 6.39(ddd, J=15.3; 11.0; 1.3Hz, 1H), 5.94(dd, J=11.0; 10.9Hz, 1H), 5.60(dt, J=15.1; 7.5Hz, 1H), 5.37(dt, J=10.9; 7.6Hz, 1H), 4.11(t, J=6.8Hz, 2H), 2.43(q, J=6.8Hz, 2H), 2.15(q, J=6.9Hz, 2H), 2.04(s, 3H), 1.42-1.24(m, 8H), 0.88(t, J=6,9Hz, 3H). ^{13}C NMR δ: (**3*E*,5*Z***) 171.0, 131.5, 128.6, 128.2, 128.1, 63.8, 32.1, 31.7, 29.6, 28.9, 27.7, 22.6, 21.0, 14.1. MS *m/z* (relative intensity); (**3*E*,5*Z***): 224(M, 0), 80(100), 79(92), 43(43), 164(38), 93(25), 78(19), 91(18), 110(10), 61(0). (**3*E*,5*E***): 224(M, 0), 80(100), 79(90), 43(30), 164(25), 41(25), 93(22), 123(5), 111(2), 61(0).

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