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Identification of sex pheromone components of the pea midge, *Contarinia pisi* (Diptera: Cecidomyiidae)

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Abstract Three components in extract of pheromone glands of female pea midges, *Contarinia pisi*, were found to be active on male pea midge antennae by coupled gas chromatographic-electroantennographic detection. The EAD active components were identified as 2-acetoxytridecane, (2S,11S)-diacetoxytridecane, and

(2*S*,12*S*)-diacetoxytridecane. A blend of these compounds proved to be highly attractive to males in windtunnel experiments.

The pea midge, *Contarinia pisi* Winn. (Diptera: Cecidomyiidae), is a serious pest reducing the yield and quality of commercial peas (Thygesen 1971; Wall et al. 1994; Pillon and Thieuleux 1995). One of the major difficulties in pea midge control is to accurately predict emergence and flight period et al. (1985) showed the presence of a female sex pheromone in the pea midge and concluded that this could be used for monitoring. Information on the chemistry of sex pheromones within the family Cecidomyiidae is scarce. (2S)-(E)-10-Tridecen-2-yl acetate has been identified in the Hessian fly, Mayetiola destructor (Foster et al. 1991). We have now identified similar compounds as sex pheromone components in female pea midges. Several tons of soil were collected in the United Kingdom and France to obtain cocoons with overwintering larvae. The cocoons were separated from the soil with water using a sieving method (Doane et al. 1987), and the adults were allowed to emerge at L/D 18/6, 25°C, and RH 70%. Hexane extracts of excised ovipositors of adult females proved to be highly attractive to males, as shown previously for calling females (Hillbur and Löfqvist 1996). The same was true for collected effluvia. Gas chromatograms of extracts and effluvia consistently showed two female specific peaks (2) and 3) which elicited clear responses in male antennae when extracts of ovipositors were analysed by coupled gas chromatographic-electroantennographic detection (GC-EAD; Arn et al. 1975; Fig. 1a). Figure 1a also shows an EAD response elicited by compound 1 eluting at an earlier retention time, where no peak in the flame ionization detector signal was visible. Analyses of EAD-active components

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Fig 1. a) Flame ionization detector (FID) and electroantennographic detector (EAD; male *Contarinia pisi* antenna) responses to a hexane extract of 20 *C. pisi* ovipositors. 1–3, EAD-active compounds. b) Mass spectra and structures of 2,11-diacetoxytridecane **2** and 2,12-diacetoxytridecane **3**

2 and **3** were carried out by GC/MS using a VG 70-250 SE mass spectrometer linked to an HP 5890 GC, with a 30 m \times 0.25 mm i.d. \times 0.25 µm film Innowax column (injection at 80° C, 2 min hold, programmed at 10° C/min to 230° C; splitless injection 1.5 min, 250° C). With a hexane extract of 100 excised ovipositors of *C. pisi* the 70 eV EI mass spectra of compounds **2** and **3** shown in Fig. 1b were obtained.

The mass spectra showed typical fragments of 2-acetoxy alkanes: m/z 43 [2: 100%, **3**: 100%; CH₃CO⁺], *m/z* 61 [**2**: 6%, **3**: 8%; CH₃C(OH)₂⁺] and m/z 87 [2: 7%, 3: 16%; $CH_3CH(OAc)^+$]. Both compounds also produced significant signals of m/z 103 [2: 14%, 3: 10%] and m/z 197 [2: 4%, 3: 3%]. Fragmentation of α -glycol diacetates produces m/z 103 representing protonated acetic acid anhydride (Sasaki 1967), which suggested the unknown pea midge compounds to be diacetates. If the fragment m/z 197 were complementary to m/z 103, both 2 and 3 would show a molecular mass of 300. This is in line with saturated tridecyl diacetates and further supported by an intensive signal at m/z180 [**2**: 12%, **3**: 12%; C₁₃H₂₄⁺], representing the parent hydrocarbon, tridecadiene, formed by double elimination of acetic acid.

While m/z 87 reveals a 2-acetoxy structure to be present in both compounds, the position of the second acetoxy group could be deduced from differences between the mass spectra. The fragment m/z 101 in the spectrum of 2 is homologous to m/z 87 and therefore suggested compound 2 to 2,11-diacetoxytridecane be [m/z] $101 = C_2H_5CH(OAc)^+$]. The higher abundance of m/z 87 in the spectrum of 3 than in that of 2, as well as the absence of m/z 101 or any other homologues of m/z 87, indicated that compound 3 was 2,12-diacetoxytridecane.

A mixture of stereoisomers of 2,11diacetoxytridecane 2 was prepared by reaction of 10-undecenal (Aldrich) with methylmagnesium bromide. The resulting 11-dodecen-2-ol was epoxidized with *m*-CPBA in dichloromethane. The epoxide was opened with methylmagnesium bromide in THF (catalyzed by 10% copper iodide) to 2,11-dihydroxytridecane, which gave diacetate 2 by acetylation with acetic acid anhydride. For the synthesis of 2,12-diacetoxytridecane 3, 1,9-dibromoheptane was converted to the bis-Grignard reagent. Addition of an excess of methyloxirane in the presence of copper iodide yielded 2,12-dihydroxytridecane, which gave diacetate 3 by acetylation with acetic acid anhydride. The synthetic compounds showed retention times and mass spectra identical with those of the female-produced compounds.

All stereoisomers of **2** and **3** could be well separated and distinguished by GC/MS using a custom made $25 \text{ m} \times 0.25 \text{ mm}$ i.d. fused silica column coated with heptakis (6-*O*-tert. butyldimethylsilyl-2,3-di-*O*-methyl)- β -cyclodextrin (König et al. 1994) at 135° C and helium as carrier gas.

The synthesis of the pure stereoisomers of 2 started with the protection of 6-bromohexanol (Aldrich) with dihydropyran. The Grignard reagent of the resulting bromide was chain elongated with optically active methyloxirane. The newly generated secondary hydroxyl group was benzylated, and the THP-protected primary alcohol was converted to the corresponding bromide (Wiley et al. 1964). The Grignard reagent of this bromide was reacted with optically active epichlorohydrine (Ohta et al. 1990) to yield 1chloro-2-hydroxy-11-benzyloxy-dodecane, showing well defined configurations at both stereocenters. Upon the action of sodium hydroxide this chlorohydrine was transformed to 1,2epoxy-11-benzyloxy-dodecane which was opened with methylmagnesium bromide. Debenzylation of the reaction product followed by acetylation yielded the desired stereoisomer of **2**. Since both (R)- and (S)-methyloxirane as well as (R)- and (S)-epichlorohydrine are commercially available

(Aldrich) all four stereoisomers of **2** could be prepared.

The synthesis of the optically active stereoisomers of 3 was even more straightforward since it followed the sequence used for the mixture of stereoisomers but employed optically active methyloxirane instead of the racemate. The synthesis of meso-3 started with 6-bromoheptanol (Aldrich), which was THP-protected at the hydroxyl group and chain elongated with optically active methyloxirane to yield 1(2-tetrahydropyranyloxy)-9-hydroxydecane. This was benzylated at the secondary hydroxyl group and further converted to the primary bromide. Subsequently this was again chain elongated with optically active methyloxirane showing the opposite configuration to that which was used first. The target isomer, meso-3, was obtained upon debenzylation and acetylation. Chiral GC proved the pure stereoisomers to show an enantiomeric excess (ee) of at least 98%. Comparison of retention times of natural and synthetic compounds using GC/MS in singleion monitoring mode proved the naturally occurring stereoisomers of 2 and **3** to show (2S,11S)-configuration and (2S, 12S)-configuration, respectively. The presence of small amounts (below 5%) of other stereoisomers cannot be excluded. The retention time of the early eluting compound 1 (no mass spectrum of the natural compound could be obtained because of its low concentration) coincided with that of 2-acetoxytridecane, which was found to be highly EAD-active. 2-Acetoxytridecane was obtained by acetylation of commercially available (Aldrich) 2-tridecanol.

All three compounds were tested in the windtunnel. A mixture of the two major components, (2S,11S)-2 and (2S,12S)-3, did not attract pea midge males; adding 1% of 2-acetoxytridecane produced a high attractancy.

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