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(2*R*,5*S*)-Theaspirane Identified as the Kairomone for the Banana Weevil, *Cosmopolites sordidus*, from Attractive Senesced Leaves of the Host Banana, *Musa* spp.

Short title: Banana weevil attractant identification

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The principal active component produced by highly attractive senesced host banana leaves, *Musa* spp., for the banana weevil, *Cosmopolites sordidus*, is shown by coupled gas chromatography-electroantennography (GC-EAG), coupled GC-mass spectrometry (GC-MS), chemical synthesis and coupled enantioselective (chiral) GC-EAG to be (2*R*,5*S*)-theaspirane. In laboratory behaviour tests, the synthetic compound is as attractive as natural host leaf material and presents a new opportunity for pest control.

The banana weevil, *Cosmopolites sordidus* Germar (Coleoptera, Curculionidae), is the most important insect pest of bananas and plantains, *Musa spp*.[1-3] throughout the world. Feeding damage is caused by larvae of *C. sordidus* which are protected within the plant tissue, and so management strategies target adult weevils. Pheromones and other semiochemicals (naturally-occurring behaviour- or development-modifying chemicals) constitute important tools for monitoring and detecting insect populations. A male-produced aggregation pheromone, (1*S*,3*R*,5*R*,7*S*)-sordidin, has been identified for *C. sordidus*.[4] For smallholder farmers in Ghana, for whom banana and plantain provide staple food, (1*S*,3*R*,5*R*,7*S*)-sordidin is deemed to be too expensive, and alternative semiochemical-based tools are urgently sought. Previous studies have shown that host plant location by adult *C. sordidus* is influenced by a highly attractive volatile kairomone from senesced banana leaves,[5,6] which, if identified, could provide an effective and affordable alternative lure for management of *C. sordidus* on smallholder farms. The purpose of this work was to identify the active component(s) from volatile material collected from senesced leaves, using coupled gas chromatography-electroantennography (GC-EAG) recordings from the antennae of adult female *C. sordidus*, and confirm the attractiveness of the identified compound(s), thereby providing the quality assurance for using senesced banana leaves as an ethnobotanically-based locally produced material in *C. sordidus* management.

10.1002/chem.201800315

Coupled GC-EAG (**ESI**) analysis with natural volatile material collected from senesced banana leaf material confirmed that the attractiveness of the material was caused by a very minor component with highly significant EAG activity (Figure 1). The 70eV EI mass spectrum of the unknown EAG-active component (Figure 2) showed a base peak at *m/z* 138, an additional diagnostic fragment at *m/z* 179 and a molecular ion at *m/z* 194. Comparison of this spectrum with the literature[7,8] suggested a theaspirane isomer **1**, the base peak being rationalised by loss formally of isobutene (C_4H_8) *via* a retro Diels-Alder rearrangement (Figure 2 inset). The presence of two stereocentres (at the 2- and 5- positions) gives four possible stereoisomers, produced initially as the mixture, by chemoenzymatic synthesis from dihydro-β-ionone **2** (Scheme). To approach resolution of the natural EAG active isomer, initial reduction of **2** with sodium borohydride in a non-stereospecific manner gave a mixture of the (*R*) and (*S*)-isomers of dihydro-β-ionol in overall 100% yield. The mixture of ionol isomers was resolved chemoenzymatically using lipase-mediated acetylation (*Pseudomonas cepaciae* lipase Amano PS-C, vinyl acetate, 99.2% ee *R*, 94.8% ee *S*). By adjusting incubation time, it was possible to obtain 99.1% ee *S*. Following separation of the (*R*)-ionol acetate and the (*S*)-ionol by silica gel liquid chromatography, the ionol then underwent intramolecular 5-*exo*-trig cyclisation upon heat treatment with selenium dioxide in dioxan to generate a diastereomeric pair of theaspirane isomers ((2*S*,5*S*)-**1**, (2*S*,5*R*)-**1**) (**ESI**), overall 35% yield over 2 steps). Cleavage of the (*R*)-acetate (using potassium hydroxide in aqueous methanol) followed by similar treatment of the (*R*)-ionol with selenium dioxide in dioxan furnished the other diastereomeric pair of theaspirane isomers ((2*R*,5*R*)-**1**, (2*R*,5*S*)-**1**) (**ESI**) in overall 41% yield over 2 steps. The diastereoisomers were difficult to separate on silica gel (4% diethyl ether in petroleum ether) due to their lack of polarity and so the isolated diastereomeric excesses were variable and mixed fractions reduced recovery. However, a purified enantiomer of the synthetic natural product, (2*R*,5*S*)-**1**, was obtained in 98.7% ee, 99.5% de. To verify the relative stereochemistry, nuclear

10.1002/chem.201800315

Overhauser experiments on the (2*R*,5*S*)-**1** showed a nOe correlation between the 6-Me groups and the H-2 proton showing this proton must be on the face of the tetrahydrofuran moiety facing to the C-6 gem-dimethyl group (**ESI**). Complementary verification was observed by analysing (5*R*,2*R*)-**1** in which a nOe correlation was observed between the 2-Me group and the C-6 gem-dimethyl group. Coupled enantioselective (chiral) GC-EAG (**ESI**) analysis using a mix of all four synthetic isomers revealed the relative GC retention times of the isomers (Figure 3, upper trace), and comparison with coupled enantioselective GC-EAG analysis using the natural volatile material collected from senesced banana leaf material revealed matching GC retention times for the (2*R*,5*S*)-isomer and the natural theaspirane isomer (Figure 3 lower trace), thus confirming the identity of the electrophysiologically active naturally-occurring isomer to be (2*R*,5*S*)-**1**.

In behaviour assays with female *C. sordidus* conducted in a linear three chamber olfactometer (**ESI**), senesced banana leaf material and collected volatile organic compounds (VOCs) were significantly more attractive ($P = 0.013$ and 0.001 respectively) than controls and were equally attractive in dual-choice assays. A mixture of the natural (2*R*,5*S*)-**1** and non-natural (2*S*,5*R*)-**1** isomers was behaviourally active at a dose of 0.2 μg and 0.02 micrograms (μg) (Students' *t*-test; $P < 0.003$, $P<0.01$ respectively). A mixture of the non-natural (2*S*,5*S*)-**1** and (2*R*,5*R*)-**1** isomers was shown to have behavioural activity only at a dose of 0.2 μg ($P = 0.04$), in spite of the observed EAG activity for (2*S*,5*S*)-**1**. A mixture of all four isomers of **1** was behaviourally active at all doses tested ie. 2 (tested twice), 0.2 and 0.02 μg ($P = 0.001$, 0.017, 0.001 and 0.002 respectively). When tested in combination with commercially available sordidin (Cosmolure), a mixture of (2*R*,5*S*)-**1** and (2*S*,5*R*)-**1** at a dose of 0.05 μg synergised the activity of the pheromone ($P = 0.04$). The EAG data suggests that antennal detection of the theaspiranes

requires a particular structural motif, ie. 5*S* stereochemistry, but that a specific overall 3D structure of the compound, ie (2*R*,5*S*), is required to elicit the behavioural response in adult female *C. sordidus*. Our data suggest that the newly identified compound (2*R*,5*S*)-**1**, present in minor quantities in senesced banana leaf material, is responsible for the attraction of adult female *C. sordidus* and is therefore the major kairomone component. The identification provides the quality assurance for the deployment of readily available senesced banana leaf material, or locally-produced extracts thereof, as a lure component of affordable trapping technology that can manage *C. sordidus* on smallholder banana and plantain farms.

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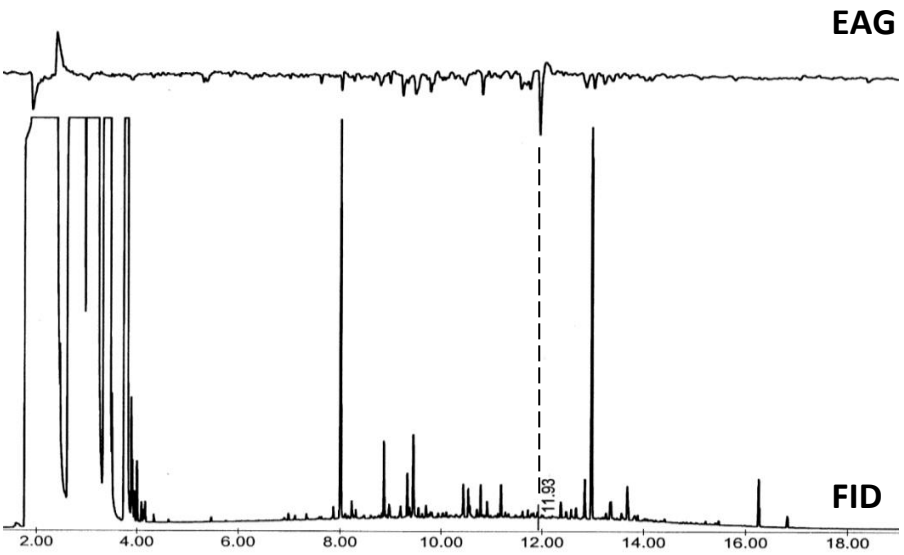
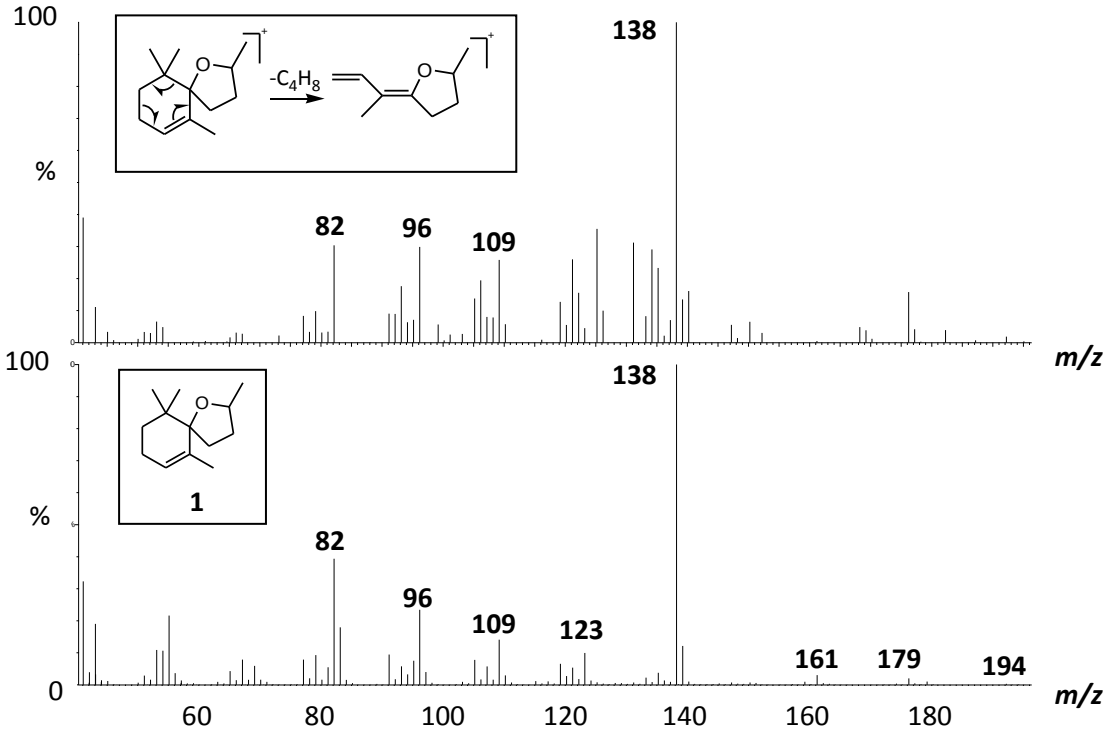
10.1002/chem.201800315

Figure 1. Coupled GC-EAG responses of adult *C. sordidus* to natural volatile material collected from senesced banana leaves volatile material collected by headspace collection, on a non-polar DB-1 GC column. The annotated peak is a minor component with major consistent EAG activity.



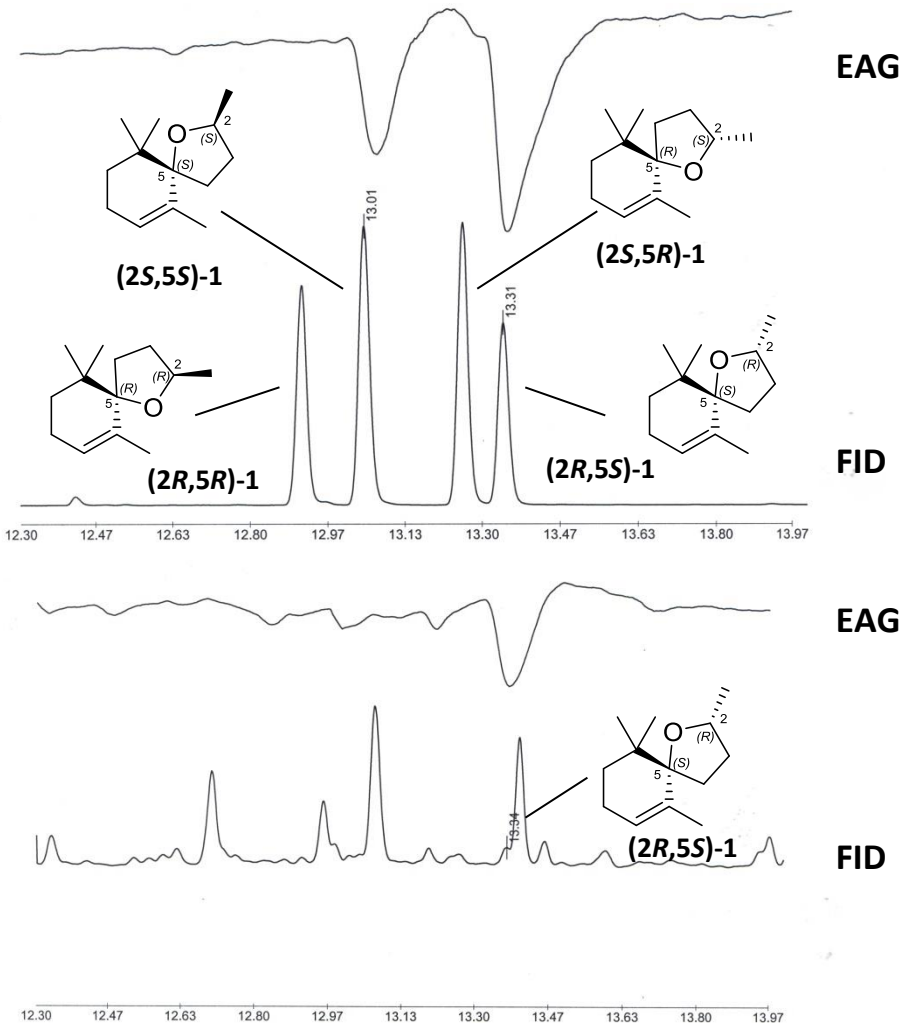
10.1002/chem.201800315

Figure 2. 70eV EI mass spectrum of EAG-active compound identified from natural volatile material collected from senesced banana leaves (upper), identified as a theaspirane isomer **1** and NIST-MS of theaspirane (lower). Inset: retro-Diels-Alder re-arrangement of parent ion from **1**.



10.1002/chem.201800315

Figure 3. Enantioselective (chiral) coupled gas chromatography-electroantennography (GC-EAG) analysis of the four synthesized theaspirane isomers (upper traces) and natural volatile material collected from senesced banana leaves (lower traces), showing alignment of the (2*R*,5*S*)-isomer **1** with the natural theaspirane isomer and the single EAG peak for the natural isomer.



10.1002/chem.201800315

Scheme. Chemoenzymatic synthesis of theaspirane isomers.