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IDENTIFICATION OF MALE-SPECIFIC CHIRAL COMPOUND FROM THE SUGARCANE WEEVIL *Sphenophorus levis*

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Abstract—Comparative gas chromatographic analyses of airborne volatiles produced by males and females of the sugarcane weevil *Sphenophorus levis*, showed one male-specific compound. Gas chromatography–mass spectrometry data indicated an aliphatic alcohol that was identified as 2-methyl-4-octanol. Both optical isomers were synthesized in five steps by employing commercially available (*R*)- and (*S*)-2,2-dimethyl-1,3-dioxolane-4-methanol as starting material. Enantiomeric resolution by gas chromatography with a chiral column demonstrated that the natural alcohol possessed the *S* configuration. Preliminary indoor observations suggested that the alcohol elicited aggregation behavior among adults. The same compound has been previously described as an aggregation pheromone in several other curculionid species.

Key Words—Sugarcane weevil, *Sphenophorus levis*, aggregation pheromone, 2-methyl-4-octanol, 3-methyl-4-octanol, *Metamasius hemipterus*.

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INTRODUCTION

Sphenophorus levis (Coleoptera: Curculionidae) is a weevil found in northern Argentina, Paraguay, and Brazil and develops mainly on sugarcane (Vanin, 1988). This species was described as a pest in 1977 and was classified as a new species in 1978 (Precetti, and Arrigoni, 1990). In Brazil it has been detected in sugarcane only in the state of São Paulo, with an endemic incidence in a southwestern region of the state (Vanin, 1988). The use of synthetic insecticides for control of this insect has been unsuccessful due to its behavior at the larval stage. During this time, similar to other species of this family, *S. levis* tunnels in sugarcane stalks, destroying tissues and weakening the plant. (Cerda et al., 1999). The increasing economic and agricultural importance of this pest in Brazil led us to investigate the feasibility of using its pheromone system for pest management.

Male-produced aggregation pheromones have been described in several curculionidae belonging to the same subfamily (Rhynchophorinae) as *S. levis* (Rochat et al., 1991; Hallett et al., 1993; Oehlschlager et al., 1995; Giblin-Davis et al., 2000). A similar species also found in Brazil, *Metamasius hemipterus*, produces six male-specific compounds (Ramirez-Lucas et al., 1996a; Perez et al., 1997) but only two of them seem to be essential as attractants (Ramirez-Lucas et al., 1996b; Perez et al., 1997). *M. hemipterus* and *S. levis* belong to the same taxonomic class; however, huge morphological and behavioral differences have been found between them.

Here we are compiling the first results of our studies on the chemical ecology of *S. levis*, reporting the identification, synthesis, and absolute configuration of a male-specific chiral compound that we believe to be the aggregation pheromone of this species.

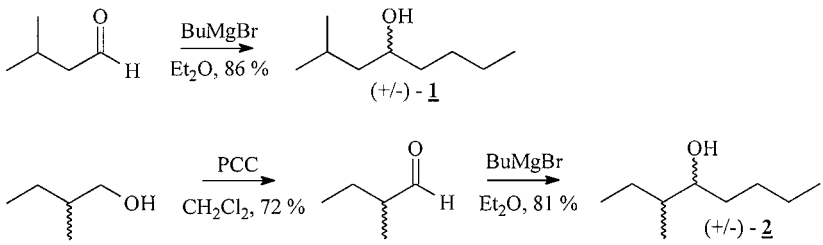
METHODS AND MATERIALS

Collection of Insect Volatiles. Adult weevils of mixed age, sex, and mating status were collected in a sugarcane plantation at the Centro de Tecnologia da Copersucar, Piracicaba, in December 1999 and January 2000. They were provided with sugarcane stalks and transported to Curitiba. Weevils were separated by sex and kept in the laboratory at 25°C under a 14 L:10 D photoperiod. Groups of 60 males and females were placed in different all-glass aeration chambers (Zarbin et al., 1998a), and the volatiles were trapped on Super Q (Alltech, Deerfield, Illinois, USA) columns for 6 days. A humidified charcoal-filtered airstream (1 liter/min) was maintained through the aeration apparatus. The columns were washed with distilled hexane, and the extracts were concentrated to 10 insect equivalents (10 IE/μL) under an argon stream in a clean conical bottom vial. (Zarbin, 2001).

General Analytical Procedures. Gas chromatographic analyses were performed on a Varian 3800 GC equipped with FID, eletronic pressure control, and operated in splitless mode. The following capillary columns were used: VA-5 (30 m $\times$ 0.25 mm $\times$ 0.25 μm), oven temperature 50°C for 3 min, programmed at 5°C/min to 150°C, held for 1 min, then rising 10°C/min to 270°C and held at this temperature for additional 10 min; and VA-Wax (30 m $\times$ 0.25 mm $\times$ 0.25 μm), oven temperature 50°C for 3 min, increased to 220°C at a rate of 7°C/min, and held for 10 min. Mass spectra were recorded on a Varian Saturn 2000 GC-MS-MS ion trap detector using the same type VA-5 capillary column under the same conditions as described above. The equipment was operated in the electron impact (EI) ionization mode (70 eV) and in the chemical ionization (CI) mode (CH_3CN). Chiral separations were obtained on a cyclodextrin-based capillary column [heptakis (2,6-di-*O*-methyl-3-*O*-phenyl)-β-CD, 20% in OV-1701, w/w, 25 m, 0.25 mm ID] using helium as carrier gas (38 psi) at 60°C. The IR spectra refer to films and were measured on a Bomem M-102 spectrometer. The ^{1}HNMR spectra were recorded with TMS as an internal standard at 400 MHz on a Bruker ARX 400 spectrometer. The ^{13}CNMR spectra were recorded with TMS as an internal standard at 100 MHz on a Bruker ARX 400. Optical rotations were measured on a Bellinghan + Stanley Ltd model D polarimeter.

Purification of Male-Specific Compound. Crude male aeration extracts were subjected to purification on silica gel columns eluted with hexane–ether mixtures of increasing polarities (100:0, 90:10, 80:20, 70:30, 50:50, 20:80, 0:100). The male-specific compound was recovered along with a small amount of other impurities in the hexane–ether 70:30 fraction. This sample was again carefully fractionated by eluting with hexane:ether mixtures (90:10, 85:15, 80:20, 75:25, 70:30, 65:35, 60:40). In the hexane–ether 75:25 fraction, the compound was isolated in good purity to be submitted to chiral analyses.

Synthesis. All reagents and solvents used in the syntheses were of highest commercially available standard. Chromatographic purifications were carried out on silica gel 60, Merck, 230–400 mesh. Racemic 2-methyl-4-octanol **(1)** (Scheme 1) was prepared by reaction of butyl magnesium bromide with



SCHEME 1. Synthesis of (±)-2-methyl-4-octanol **(1)** and (±)-3-methyl-4-octanol **(2)**.

isovaleraldehyde in ether solution at 0°C. Standard work up yielded **(1)** in 86% (Perez et al., 1997). Racemic 3-methyl-4-octanol **(2)** and 5-nonanol were synthesized using the same conditions (as described above, employing 2-mehtylbutanal [prepared by oxidation of 2-mehyl-1-butanol (Aldrich) with PCC in dichloromethane, 72% yield (Zarbin et al., 1998b)] and butanal, respectively [81% yield for **(2)** and 74% yield for 5-nonanol].

*(*4R*)- and (*4S*)-2,2-Dimethyl-4-Toluene-4-Sulfonyloxymethyl 1,3-Dioxolane **(4)***. Tosyl chloride (2.07 g, 10.88 mmol) was added in small portions (1 hr) to a solution of alcohol **(3)** (1.20 g, 9.08 mmol; *R* or *S*), triethylamine (1.38 g, 13,61 mmol, 1.90 ml), and DMAP (112 mg, 0.09 mmol) in dry dichloromethane (20 ml), with magnetic stirring at 0°C. After 5 hr, ether was added, and the solution was washed with aqueous HCl (10%), saturated aqueous $NaHCO_3$, dried over $MgSO_4$, and concentrated *in vacuo*. The crude product was chromatographed over silica gel (hexane–ethyl acetate, 5:1), and the product **(4)** was obtained in 88% yield (2.2 g). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.30 (s, 3H); 1.35 (s, 3H); 2.45 (s, 3H); 3.76 (dd, $J = 5.1$, $J = 10.7$ Hz, 1H); 3.94–4.09 (m, 3H); 4.26 (dq, $J = 5.1$, $J = 6.1$ Hz, 1H); 7.35 (d, $J = 6.1$ Hz, 2H); 7.80 (d, $J = 6.1$ Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 21.66; 25.14; 26.64; 66.19; 69.60; 72.92; 110.07; 128.00; 130.01; 132.66; 145.00. {(4*S*)-**(4)**, $[\alpha]_D^{25} = +4.3$ (*c* 1.0, EtOH); (4*R*)-**(4)**, $[\alpha]_D^{25} = -4.6$ (*c* 1.2, EtOH)}.

*(*4R*)- and (*4S*)-4-Butyl-2,2-Dimethyl-1,3-Dioxolane **(5)***. Propylmagnesium bromide (5 eq. in THF) and $CuBr \cdot Me_2S$ (1.4 mmol, 286 mg) were added to a solution of **4** (2.0 g, 6.70 mmol) in dry THF (70 ml) at −40°C under an argon atmosphere. The temperature was gradually raised to room temperature and the reaction was stirred for 24 hr. Subsequently, 10 ml of saturated NH_4Cl was added, and the organic layer was diluted with 60 ml of ether. The extract was washed with water (5 ml), saturated $NaHCO_3$, and saturated NaCl. The layers were separated, and the organic layer was dried ($MgSO_4$) and concentrated *in vacuo*. The residue was subjected to column chromatography (hexane–ethyl ether, 3:1) on silica gel to yield 0.61 g (55.6 %) of **(5)**. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.91 (t, $J = 7.0$ Hz, 3H); 1.14–1.59 (m, 5H); 1.37 (s, 3H); 1.42 (s, 3H); 1.60–1.71 (m, 1H); 3.50 (t, $J = 7.2$ Hz, 1H); 4.01–4.11 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$)δ: 14.04; 22.75; 25.77; 26.96; 27.94; 33.30; 69.55; 76.17; 108.56. {(4*S*)-**(5)**, $[\alpha]_D^{25} = +20.1$ (*c* 1.45, $CHCl_3$); (4*R*)-**(5)**, $[\alpha]_D^{25} = -20.5$ (*c* 1.32, $CHCl_3$)}.

*(*2R*)- and (*2S*)-2-Hexanediol **(6)***. To a mixture of methanol (5 ml) and 2 N HCl (5 ml) was added dropwise compound **5** (0.54 g; 3.44 mmol) in methanol (1 ml). The solution was stirred for 2 hr at room temperature, and then a saturated aqueous solution of K_2CO_3 was slowly added at 0°C. Ether (50 ml) was added, and the organic layer was washed with saturated NaCl solution, separated, dried over $MgSO_4$, and concentrated *in vacuo*. The residual oil was chromatographed on a silica gel column (hexane; ethyl ether, 1:1) to afford 0.4 g of **(6)** (99 %). IR (ν_{max}, film cm^{-1}): 3338; 2932; 2861; 1466; 1062. ^{1}H NMR (400 MHz,

$CDCl_3$) δ: 0.91 (t, $J = 7.1$ Hz, 3H); 1.25–1.50 (m, 6H); 2.85 (bs, 2H); 3.42 (dd, $J = 7.7$, $J = 11.2$ Hz, 1H); 3.62-3.73 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 14.01; 22.75; 27.79; 32.80; 66.76; 72.40. GC-MS (70 eV) m/z (%): 117 (M-1, 2); 101 (14); 83 (100); 69 (77); 55 (8). {(2*S*)-**(6)**, $[\alpha]_D^{25} = +2.0$ (*c* 3.38, $CHCl_3$); (2*R*)-**(6)**, $[\alpha]_D^{25} = -2.17$ (*c* 9.5, $CHCl_3$)}.

*(*2R*)- and (*2S*)-2-Butyloxirane **(7)***. The diol **(6)** (0.39 g, 3.30 mmol) was added to a suspension of KOH (1.10 g, 19.50 mmol) in dry ethyl ether (10 ml) at 0°C, followed by slow addition of an ethereal solution (5 ml) of tosyl chloride (0.65 g, 3.4 mmol). The mixture was stirred for 2 hr at room temperature. After addition of hot water (5 ml), the mixture was extracted with ether (3 × 10 ml), the organic layer was separated, dried over $MgSO_4$, and the solvent was carefully removed by distillation. The residue (0.12 g, 37%) was employed in the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.92 (t, $J = 7.3$ Hz, 3H); 1.32–1.58 (m, 6H); 2.46 (dd, $J = 2.8$, $J = 5.1$ Hz; 1H); 2.74 (dd, $J = 4.0$, $J = 5.1$ Hz, 1H); 2.90 (ddt, $J = 2.8$, $J = 4.0$, $J = 5.6$ Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 13.99; 22.56; 28.12; 32.21; 47.11; 52.39. {(2*S*)-**(7)**, $[\alpha]_D^{25} = -9.3$ (*c* 1.20, $CHCl_3$), [Lit: $[\alpha]_D^{20} = -10.1$ (*c* 1.53, $CHCl_3$); Francke et al., 1995); (2*R*)-**(7)**, $[\alpha]_D^{25} = +9.0$ (*c* 10.5, $CHCl_3$)}.

*(*4R*)- and (*4S*)-2-Methyl-4-Octanol **(1)***. To a stirred solution of **7** (0.27 g, 2.7 mmol) in dry THF (5 ml) kept below −40°C, was added CuI (0.52 g, 2.7 mmol) followed by dropwise addition of PrMgBr (2M solution in ether, 13.5 ml, 27 mmol). After the addition was completed, the mixture turned dark. The mixture was allowed to warm to room temperature, and stirring was continued for 1 hr. The reaction was quenched by addition of saturated NH_4Cl (10 ml). The aqueous layer was extracted with hexane–ethyl ether, 1:1 (3 × 20 ml), and the organic layer was dried over $MgSO_4$ and concentrated carefully *in vacuo*. The product was purified by flash chromatography on silica gel using a mixture of hexane–ethyl ether, 5:1, yielding compound **1** as a colorless oil (0.32 g, 82 %). IR (ν_{max}, film cm^{-1}): 3346; 2956; 2928; 2870; 1466; 1026. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.90 (d, $J = 6.6$ Hz, 3H); 0.91 (t, $J = 7.0$ Hz, 3H); 0.92 (d, $J = 6.6$ Hz; 3H); 1.19–1.48 (m, 8H); 1.70 (bs, 1H); 1.71–1.81 (m, 1H); 3.64–3.72 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 14.50; 22.46; 23.17; 23.91; 25.03; 28.22; 38.19; 47.22; 70.40. {(4*S*)-**(1)**, $[\alpha]_D^{25} = +9.17$ (*c* 2.3, $CHCl_3$), 92% *ee* by chiral GC analyses, $R_t = 7.81$ min; (4*R*)-**(1)**, $[\alpha]_D^{25} = -9.21$ (*c* 1.5, $CHCl_3$), 93% *ee* by chiral GC analyses, $R_t = 7.35$ min}.

RESULTS AND DISCUSSION

Gas chromatographic (GC-FID) analysis of the airborne volatiles produced by males and females of the sugarcane weevil *S. levis* showed the presence of one male-specific compound (Figure 1). The base peak observed on GC-EI mass

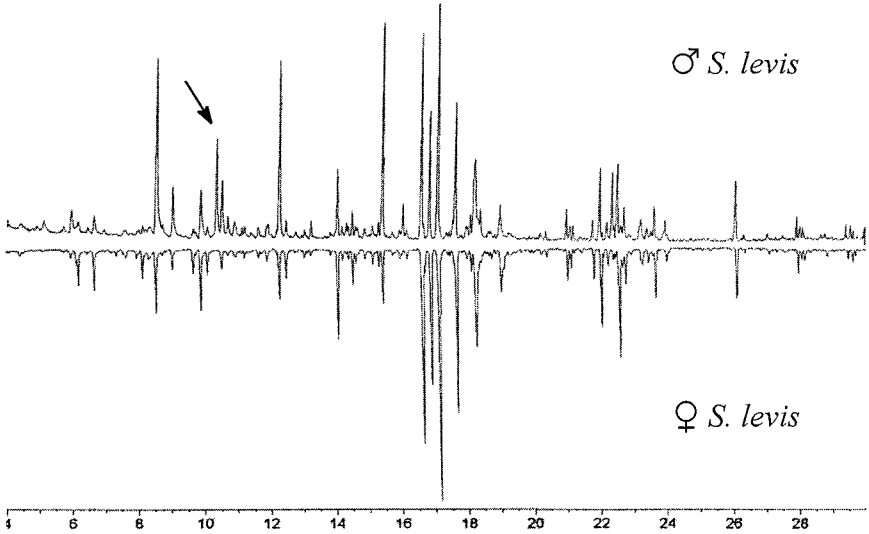


FIG. 1. GC profiles of the extracts of airborne volatiles collected from male and female sugarcane weevils *S. levis*. The male-specific compound is marked by an arrow.

spectra (MS) at m/z 69 along with the intense peak at m/z 87 strongly suggested the existence of a hydroxyl group attached to a five-carbon fragment. The m/z 126 was deduced to be due to a M−18 peak, resulting from loss of water, indicating a possible molecular ion (M^+) at m/z 144 (Figure 2). However, even employing chemical ionization with CH_3CN, the molecular ion of the compound could not be detected. The hypothesis that the target molecule was a linear and symmetrical structure such as 5-nonanol was ruled out, since the MS of the compound gave an ion at m/z 111, typical M−33 of methyl branched alcohol that results from loss of CH_3 and H_2O (Silverstein et al., 1991). Moreover, the retention time (R_t) of

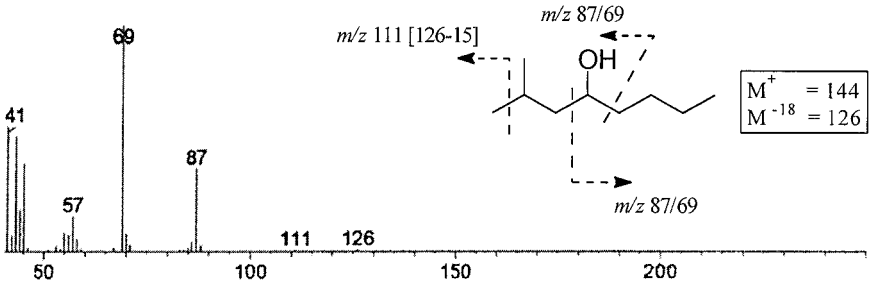
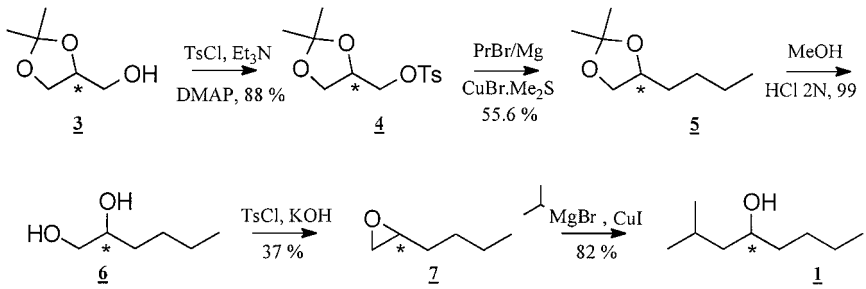


FIG. 2. Mass spectra of the male-specific compound of *S. levis*. Inset: major fragmentation patterns of the molecule.

a synthetic sample of 5-nonanol was higher than that of the natural product. At this point, the data suggested only two possible chemical structures for this compound; 2-methyl-4-octanol **(1)** or 3-methyl-4-octanol **(2)**. In order to determine unambiguously the correct structure of this male-specific compound, both alcohols were synthesized as racemates and used as authentic references (Scheme 1). The mass spectrometric characteristics of both standards were identical to those of the natural product, but only the 2-methyl-4-octanol **(1)** exhibited identical retention times on two different GC columns (VA-5 and VA-Wax). Thus, the chemical structure of the male-specific compound was deduced as 2-methyl-4-octanol.

While the carbon skeleton was determined, nothing was known about the configuration of the stereogenic center. One way to investigate the stereochemistry of chiral volatiles is to obtain enantiomeric resolution on a chiral column. The assignments of the peaks can be carried out with samples of known stereochemistry, and the absolute configuration of the natural product is determined by comparison of its retention time under the same conditions (Leal et al., 1996).

In order to determine the absolute configuration of naturally occurring **1**, we developed a short enantioselective synthesis for both enantiomers, as described in Scheme 2. Our route utilized commercially available (*R*)- and (*S*)-2,2-dimethyl-1,3-dioxolane-4-methanol **(3)** as a starting material, which were transformed into the tosylates **(4)** in 88% yield (Perkins et al., 1992). Compounds **(5)** were obtained by coupling tosylates **(4)** with *n*-propyl magnesium bromide, catalyzed by $CuBr \cdot Me_2S$, in 55.6% yield (Zarbin et al., 2000). Hydrolysis of ketals **(5)** under acidic conditions furnished the diols **(6)** in 99% yield. The highly volatile epoxides **(7)** were obtained in "one pot" from diols **(6)** in 37% isolated yield, by reaction with tosyl chloride and powdered KOH (Francke et al., 1995; Jurczak et al., 1986; Kuwahara et al., 1997). Finally, the epoxides **(7)** were reacted with 1-methylethyl magnesium bromide catalyzed by CuI, affording the target compounds **(1)** in 82% yield (Takenaka et al., 1996; Baraldi et al., 2002).



SCHEME 2. Enantioselective synthesis of (*R*)- and (*S*)-2-methyl-4-octanol **(1)**.

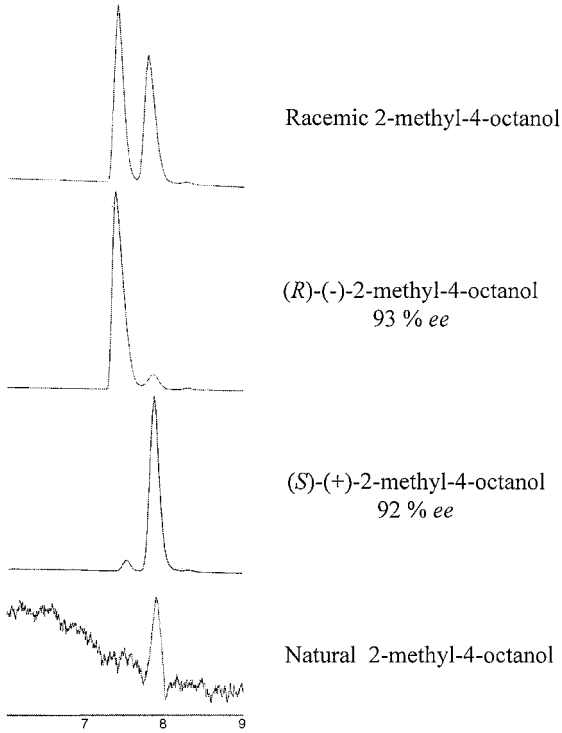


FIG. 3. Resolution of enantiomers of 2-methyl-4-octanol **(1)** on a β-CD column. The peaks correspond to7.35 and 7.81 min.

Racemic **(1)** was resolved almost to baseline on a β-CD capillary column, showing two peaks with retention times of 7.35 and 7.81 min (Figure 3). Synthetic (*R*)-**(1)** corresponded to the earlier eluting peak, while the (*S*)-**(1)** isomer gave a peak coeluting with the later one. The purified natural product had the same retention time as the second peak. Therefore, the male-specific compound produced by the sugarcane weevil *S. levis* was fully characterized as (*S*)-(+)-2-methyl-4-octanol.

At present, the biological role of the compound identified in this work is unknown or, at least, uncertain. However, preliminary laboratory observations employing rubber septa as releaser suggest that the racemic compound elicits aggregation behavior among both males and females of this species. As already mentioned, the same compound has been identified as a male-produced aggregation pheromone in several other curculionidae species (Perez et al., 1997; Ramirez-Lucas et al., 1996a,b; Giblin-Davis et al., 2000). A detailed study concerning the biological activity of racemic, as well as (*R*)- and

(*S*)-2-methyl-4-octanol, including the electrophysiological measurements, is now in progress.

In summary, we have identified a male-specific chiral compound (possibly an aggregation pheromonce) produced by the weevil *S. levis*, an important sugarcane pest in South America.

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