SEX PHEROMONE OF THE SCARAB BEETLE *Phyllophaga elenans* AND SOME INTRIGUING MINOR COMPONENTS

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Abstract—Three amino acid-derived compounds were identified in extracts from the pheromone glands of the scarab beetle *Phyllophaga elenans*, i.e., L-isoleucine methyl ester (LIME), N-formyl L-isoleucine methyl ester (For-LIME), and N-acetyl L-isoleucine methyl ester (Ac-LIME). The compounds were characterized from their spectral data (MS and IR), confirmed by synthesis, and their absolute configurations were assigned by gas chromatography with a chiral phase column. The amount of LIME in calling females was ca. 2 µg/gland, whereas only traces of For-LIME and Ac-LIME (0.005% of LIME) were detected in fresh extracts. Regardless of the storage temperature, the amounts of the minor constituents in the extracts increased over time. Field tests showed that traps baited with For-LIME captured more beetles than control traps. Ac-LIME per se was not attractive, and it did not increase trap catches when combined with For-LIME. Traps baited with LIME caught ca. 150 beetles/trap/day, but catches

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did not increase with the addition of For-LIME and/or Ac-LIME in binary or tertiary blends.

**Key Words**—Phyllophaga elenans, Holotrichia parallela, Scarabaeidae, Melolonthinae, L-isoleucine methyl ester, N-formyl L-isoleucine methyl ester, N-acetyl L-isoleucine methyl ester, chiral resolution.

**INTRODUCTION**

We have been studying chemical communication in scarab beetles (Coleoptera: Scarabaeidae), which utilize a wide variety of chemical compounds as pheromones (Leal, 1998a, 1999; Ruther et al., 2001). While pheromones of rutelines (subfamily Rutelinae) arise largely by transformation of fatty acids (Leal et al., 1999), melolonthine (Melolonthinae) pheromones are derived from fatty acids and other precursors, including isoprenoids and even amino acids (Leal, 1999). An amino acid-derived pheromone, L-isoleucine methyl ester (LIME), was first isolated and identified in the large black chafer, Holotrichia parallela (Motschulsky) (Leal et al., 1992, 1993). Later, L-isoleucine and L-valine methyl esters were identified in the cranberry white grub, Phyllophaga anxia (Zhang et al., 1997).

The genera Holotrichia and Phyllophaga belong to the same tribe Melolonthini; their separation is based on a geographical division where species from Asia are placed in the former and species from South, Central, and North America in the latter (Mila Coca Abia, personal communication). We envisaged that other Holotrichia species would utilize amino acid-derived pheromones, but to our surprise the pheromone of a species native to India, *H. consanguinea* Blanchard, was identified as anisole (Leal et al., 1996). This finding prompted us to analyze the pheromone chemistry of *Phyllophaga* species from South and Central America to compare their pheromones. Here, we report the identification of the sex pheromone of *P. elenans* (Saylor), one of the most damaging pests of grain crops and sugarcane in Central America (King, 1984).

**METHODS AND MATERIALS**

**Pheromone Extraction.** Insects were collected in Turrialba and Guanacaste, Costa Rica, or obtained from laboratory cultures maintained in Turrialba. Abdominal tips of calling females were excised with forceps, and the material was cleaned to remove tissues other than the ball-shaped sac housing the pheromone glands (Kim and Leal, 1999). Glands were extracted for 3 min either with diethyl ether or methanol. After removing the pheromone glands, samples were transferred to glass ampoules, sealed, and shipped to Tsukuba, Japan, or Davis, California. Diethyl ether was distilled in an all-glass distillation apparatus, and methanol was HPLC grade (Merck). Distilled diethyl ether was sent to Costa Rica.