(Z)-11-EICOSEN-1-OL, AN IMPORTANT NEW PHEROMONAL COMPONENT FROM THE STING OF THE HONEY BEE, *Apis mellifera* L. (HYMENOPTERA, APIDAE.)

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Abstract—(Z)-11-Eicosen-1-ol was identified by GC-MS and microchemical methods as a major volatile component, ca. 5 μg per insect, secreted by the sting apparatus of the worker honey bee. When presented on moving lures at the hive entrance, (Z)-11-eicosen-1-ol, like isopentyl acetate already known as an alarm pheromone, elicited stinging, and together these two compounds were as active as the natural pheromone from the sting. On stationary lures, (Z)-11-eicosen-1-ol prolonged the effectiveness of isopentyl acetate.


INTRODUCTION

A synthetic mixture of the Nasonov pheromone of the honey bee (Pickett et al., 1980) attracts foraging honey bees as well as equivalent amounts of natural pheromone (Williams et al., 1981). However, the electroantennographic (EAG) response to the natural extract is greater than that to an equivalent amount of synthetic pheromone (Williams et al., 1982), suggesting that the natural extract was contaminated by components of other pheromones. The sting is close to the Nasonov gland and contamination of Nasonov secretion extracts by low-molecular-weight components from the sting does occur (Pickett et al., 1980). In this work such components with higher molecular weight were sought, without assuming that they were from the sting.
Methods and Materials

Extraction and Fractionation. Worker honey bees (300) were killed by chilling at $-10^\circ$C and extracted with hexane ($2 \times 200$ ml) for 3–4 min. The filtered extract was dried ($\text{MgSO}_4 \cdot \text{H}_2\text{O}$), concentrated (20 ml) at the pump, and placed on a column of Florisil ($32 \times 300$ mm) in hexane. The column was eluted with hexane (825 ml), 20% diethyl ether (400 ml), and 50% diethyl ether (400 ml) in hexane, and portions of effluent (25 ml) were monitored by gas chromatography (OV-17). Fractions giving peaks were concentrated (250 $\mu$l) under vacuum and stored in glass ampoules under $\text{N}_2$.

Instrumental Analysis. Gas chromatography (GC) was by flame ionization (Pye 104) using glass columns, 5 ft $\times$ 0.25 in., 3% OV-17 or 2.5% OV-101, on Diatomite C AW DMCS, 80–100 at 200$^\circ$ C. Approximate amounts of (Z)-11-eicosen-1-ol were determined by comparison with peak areas from GC of standard solutions. Gas chromatography coupled with mass spectrometry (GC-MS) employed a glass capillary column, 50 m $\times$ 0.25 mm, wall coated with heat-treated Carbowax 20M (PhaseSep) at 200$^\circ$C linked directly to the source of the mass spectrometer (MM 70-70F + Data System 2025, V.G. Micromass) with electron impact ionization at 70 eV, 200$^\circ$ C.

Chemical Analysis. Portions (10 $\mu$l) of the concentrated column effluent were treated with (1) acetic anhydride (2 $\mu$l), (2) N-trimethylsilylimidazole (Tri-Sil Z, Pierce, 2 $\mu$l), and (3) m-chloroperbenzoic acid (1 mg) in chloroform (30 $\mu$l) followed by evaporation to dryness under vacuum and extraction with hexane (10 $\mu$l). The products were analyzed by GC and GC-MS. Ozonolysis was by the method of Beroza and Bierl (1967) on concentrated total extract (20 honey bee equivalents) and analysis by GC (OV-101, 50$^\circ$ 10 min, 4$^\circ$/min to 200$^\circ$ C).

Synthetic Compounds. Heneicosane, tricosane, pentacosane, (Z)-11-eicosen-1-ol, (Z)-9-octadecen-1-ol (oleyl alcohol), and (E)-9-octadecen-1-ol (elaidyl alcohol) were obtained from commercial sources and epoxides were prepared conventionally using m-chloroperbenzoic acid. Structures of alcohols and epoxides were confirmed by $^{13}$C nuclear magnetic resonance (NMR) spectroscopy (Jeol-PFT-100), in CDCl$_3$, Me$_4$Si as standard ($\delta = 0.00$), or MS and purity was established (99+%') by GC. Isopentyl acetate (3-methylbutyl ethanoate 99.9%) was purchased uncontaminated with the 2-methyl isomer (Cambrian Chemicals).

Dissection of Honey Bees. Worker honey bees (20), killed by chilling at $-10^\circ$C, were dissected into head, thorax, and abdomen, the legs were removed from the thorax, and the abdomen separated into the sting apparatus, gut, and anterior and posterior abdominal halves. Parts were placed in seven vials and crushed under hexane (400 $\mu$l). A portion (2 $\mu$l) of each extract was analyzed by GC for the presence of (Z)-11-eicosen-1-ol.