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Exocrine Secretions of Bees IX. Aliphatic Esters in the Dufour's Gland Secretion of *Synhalonia hamata*#

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ABSTRACT

Chemical analysis of Dufour's gland extracts of the eucerine bee, *Synhalonia hamata,* showed the presence of four acetates. Octadecyl acetate was the major component of the secretions and the C_{16}, C_{20}, and C_{22} acetates were also present. This combination of chemicals in the Dufours gland blend of *S. hamata* is unique.

Introduction

The Anthophoridae is a large, diverse family of widely distributed bees. One of its tribes, the Eucerini, is represented in the United States by approximately 220 species distributed among 15 of the 18 genera represented in North America.[1] *Melissodes* is the largest eucerine genus with close to 100 species represented in North America north of Mexico. *Synhalonia,* another large eucerine genus, is represented in North America north of Mexico by approximately 60 species.[1] They fly primarily in spring and are rarely observed during the summer. Most *Synhalonia* are large, robust, fast flying bees that are native to western USA.

Several studies have provided information on the chemistry of the Dufour's gland secretions of the Eucerini. Batra and Hefetz[2] reported a series of acetates including *n*-tetradecyl acetate, dihydrofarnesyl acetate and an isomer of farnesyl acetate of unknown structure in the Dufour's extracts of *Melissodes desponsa* Smith. In comparison, the Dufour's secretions of *Svastra obliqua obliqua* (Say) contain a complex mixture of 32 aliphatic esters. These esters range from a molecular weight of 256 (octyl octanoate) to 508 (tetracosyl decanoate). A series of saturated and unsaturated hydrocarbons ranging from C_{21} to C_{31} were also identified.[3]

As part of continuing comparative studies of the evolution of the chemistry, morphology, and function of the Dufour's

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gland, we describe the chemistry of the Dufour's gland secretions of *Synhalonia hamata* (Bradley).

Materials and Methods

Synhalonia hamata were collected at the Marine Training Base at Quantico, Virginia, during June and July, 1983. Bees were netted as they gathered nectar and pollen from *Penstemon digitalis* Nutt. Individual specimens were placed in separate glass shell vials and stored in an ice chest. Dufour's glands were excised under water with forceps, and groups of 25 of them were extracted with methylene chloride.

Extracts were analyzed using a Finnigan 3200 computerized gas chromatograph-mass spectrometer (GC-MS) utilizing a 2.0-m-x-1-mm 3% OV-17 on a Supelcoport 60/80 column, temperature programmed from 60 to 300°C at 10°C/min. Each compound was identified by comparing its mass spectrum and retention time with that of a standard synthesized from the corresponding alcohol and acetic anhydride in the presence of sodium acetate.

Results

One major peak was observed in the Dufour's gland secretion of *S. hamata.* Three minor peaks were also present, one eluting before the major peak and the other two following it. All four were acetates, based upon their base peak at m/z 43 and a peak of m/z 61. Comparison of the retention times and mass spectra of these acetates with those of standard synthetic samples indicated that the major component was octadecyl acetate, proceeded by hexadecyl acetate, and followed by eicosyl acetate and docosyl acetate. Besides the base peak at m/z 43 and the peak at 61(45), peaks at m/z 312(0.1), 252(3), 224(2), 196(1), 168(2), 153(2), 125(20), 111(30), 97(55), 83(65), 69(60), 57(60), 55(65), and 41(35) were useful in the assignment of the structure of octadecyl acetate. Similar peaks were used to assign other acetates.

Discussion

The Dufour's gland secretions of the Colletidae[4] and Halictidae[5] are characterized by series of saturated and unsaturated macrocyclic lactones, as well as isopentenyl esters in some species of the Halictidae.[6] Macrocyclic lactones also characterize the Dufour's gland of oxaeids.[7] In contrast, the Dufour's gland secretions of the andrenids are characterized by terpenoid and straight chain aliphatic esters.[8,9] Those of the Melittidae contain monounsaturated alcohols as well as a series of acetates and butanoates.[10] For a review of the chemistry of bee Dufour's glands, see Duffield *et al.*[11]

In the Anthophoridae, the Dufour's gland chemistry has been investigated in four genera representing two subfamilies. The Dufour's glands of *Xylocopa virginica texana* Cresson and *X. micans* Lepeletier (Xylocopinae: Xylocopini) contain a series of saturated and unsaturated hydrocarbons.[12,13] A series of triglycerides has been identified in *Anthophora abrupta* Say (Anthophorinae: Anthophorini).[14] The Dufour's extract of *Melissodes desponsa* (Anthophorinae: Eucerini) contains terpenoid and straight chain acetates,[2] whereas the Dufour's glands of *Svastra obliqua obliqua* (Eucerini) contain a complex mixture of 32 aliphatic esters as well as saturated and unsaturated hydrocarbons.[3]

At present, the Dufour's gland chemistry of *S. hamata* appears to be distinct from other bees. Although acetates are not uncommon as natural products of bees, the alcohol portion in *S. hamata* is longer than in most. For example, a series of acetates (C_8-C_{14}) has been identified in the cephalic extracts of several species of *Andrena* (Andrenidae).[15] Similar acetates (C_4-C_{10}) have been isolated from the sting apparatus of worker honey bees, *Apis mellifera* Linn. (Apidae),[16] where they function as alarm releasers. Octyl acetate has been isolated

from the worker sting apparatus in all four species of *Apis*.[17] Hexadecyl, octadecyl, and eicosyl acetates have been isolated previously from male labial glands of several species of European bumble bees.[18–21] The C_{16} acetate has also been isolated from male mandibular gland extracts of the small carpenter bee, *Ceratina cucurbitina* Rossi (Xylocopinae: Ceratini).[22]

Acetates appear to be a common group of compounds found as glandular products of bees. They have been isolated from mandibular glands, labial glands, sting glands, and Dufour's glands. The four acetates isolated from *S. hamata* appear to represent a unique Dufour's gland blend among bees reported in the literature.

The functions of acetates as glandular products of bees are diverse. Male labial gland secretions of bumble bees are used as territorial markers.[18] In contrast, the sting shaft glandular secretions of worker honey bees function as alarm pheromones.[16] The mandibular gland products of *Ceratina* appear to be effective defensive allomones against ants.[22]

It has been demonstrated that the Dufour's gland secretions of bees are used to line the brood cells in Andrenidae,[23] Anthophoridae,[14] Colletidae,[24] and Halictidae.[6] Norden *et al.* have observed *Anthophora* larvae ingesting their cell wall linings.[14] Dufour's components have been identified in the larval pollen and nectar provisions of *Augochlora pura pura* Say (Halictidae).[6] Many authors believe the Dufour's gland secretions have some antimicrobial activity, thus increasing larval survival, as discussed by Cane *et al.*[25] We are presently investigating the functions of the acetates in the Dufour's gland secretions of *Synhalonia hamata*.

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