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CASTE-SPECIFIC ESTERS DERIVED FROM THE QUEEN HONEY BEE STING APPARATUS

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Abstract—1. The sting apparatus of the queen honey bee (*Apis mellifera*) contains a series of aliphatic hydrocarbons and novel long-chain esters.

2. Decyl decanoate, the main ester present, is accompanied by decyl octanoate, dodecyl decanoate, tetradecyl decanoate, hexadecyl decanoate and tetradecyl dodecanoate.

3. The presence of these esters did not inhibit oviposition by the queen in worker cells or result in sealed queen cells being destroyed by workers.

4. The significance of caste-specific compounds on the sting of both the queen and worker honey bee is discussed.

INTRODUCTION

In the honey bee *Apis mellifera*, caste-specific compounds are characteristic of both workers and queens. For example, queens produce queen substance, 9-oxo-(*E*)-2-decenoic acid in their mandibular glands (Butler *et al.*, 1961) whereas workers produce 10-hydroxy-(*E*)-2-decenoic acid in the same glands (Butenandt and Rembold, 1957). Another possible example of caste-specific compounds was described by Maschwitz (1964) in his study of sting-derived alarm phermones of worker honey bees. Compounds on the sting shaft of a guard (worker) bee function as a powerful releaser of alarm behavior and it has been subsequently determined that this activity is identified with a large series of short-chain acetates and alcohols present on this defensive organ (Blum *et al.*, 1978). On the other hand, since the sting apparatus of the queen bee possesses no alarm-releasing activity (Maschwitz, 1964), it is possible that the queen does not generate alarm pheromones.

In the present paper we report on the chemistry of the sting apparatus of the queen bee and demonstrate that a series of novel, and caste-specific, esters are produced. In addition, a discussion of the possible significance of the caste-specific compounds produced by queens and workers is presented.

MATERIALS AND METHODS

Three groups of sting shafts, devoid of the sting-associated glands, were dissected from 1, 8 and 14 day old queens and were placed in methylene chloride.

Extracts were analyzed by gas chromatography–mass spectrometry on a LKB 2091 instrument utilizing a 25 m × 2 mm capillary column of 10% SP-1000 programmed from 60 to 230°C at 10°/min.

The possible pheromonal roles of queen-specific esters in either inhibiting oviposition by the queen in empty worker cells or designating sealed queen cells as expendable were determined by applying 2.5 μg mixtures of the esters in 2.5 μl of paraffin oil to both kinds of cells. The subsequent reactions of both queens and workers to the marked cells were monitored after treatment.

RESULTS

Chemistry of the sting shaft

Two series of compounds were identified as characteristic products of the sting shaft of the queen bee. The major constituents present consisted of a series of odd-membered *n*-alkanes dominated by compounds in the range C_{23}–C_{27}. These hydrocarbons were accompanied by six novel esters which constituted relatively minor constituents *vis-à-vis* the alkanes.

The major ester present contained a molecular ion at *m/z* 312. The acidic and alcoholic moieties of the ester were readily attributed to fragments at *m/z* 173 and 142, respectively. The fragment at *m/z* 173 arises from the acid (decanoic) +1, whereas the fragment at *m/z* 142 is derived from the alkene (decene) of the alcoholic moiety of the ester. Both the retention time and mass spectrum of the bee-derived ester were identical to those of decyl decanoate, prepared from the acid chloride of decanoic acid and decanol.

Three of the other esters also contained decanoic acid as the acidic moiety. Molecular ions at *m/z* 340, 386 and 396 indicated that a homologous series of decanoates was present. and important ions at *m/z* = 168 (dodecene), 196 (tetradecene), and 224 (hexadecene) established their structures as dodecyl decanoate, tetradecyl decanoate, and hexadecyl decanoate, respectively. The synthetic esters possessed retention times and mass spectra identical to those of the bee-derived esters.

Two other esters were also identified as sting-shaft constituents. A minor constituent was identified as decyl octanoate based on its molecular ion (m/z = 284) and significant fragments at m/z 145 and 142. The final ester to elute possessed a molecular ion at m/z 396 and diagnostic fragments at m/z 201 and 196 and was identified as tetradecyl dodecanoate. Comparison with authentic samples of these two esters confirmed their identities.

Bioassay of treated worker and queen cells

Queen bees oviposited readily in empty worker cells to which a blend of synthetic esters had been added at the base of each cell. Sealed queen cells which were treated externally with the esters elicited no responses from the worker bees and the queens emerged normally.

DISCUSSION

From a natural products standpoint, queen bees have diverged considerably from their workers. The esters produced on the sting apparatus of the queen contrast markedly with the short-chain esters (e.g. isopentyl acetate) that are present on the sting shaft of the workers (Boch *et al.*, 1962; Blum *et al.*, 1978). These biochemical differences undoubtedly have behavioral correlates in terms of the functions of the compounds produced by members of each caste. The sting-derived compounds produced by the workers have been evolved to function as releasers of alarm behavior (Collins and Blum, 1982) whereas the waxy esters produced on the sting of the queen have not been demonstrated to possess a communicative function. However, it is possible that these chemical differences are related to the fate of the sting after aggressive encounters by either queens or workers.

Queen bees can sting repeatedly, and it is possible that the waxy esters on the sting function as a lubricant that enables the queen to facilely withdraw the sting from tissues. Thus, these distinctive esters may not possess any communicative role but rather may function by promoting the hyperdermic role of the sting. On the other hand, the worker sting cannot be readily withdrawn from tissues and remains impaled after the bee has pulled its body from the sting site. Significantly, the impaled sting continues to release alarm pheromones and thus serves to orient excited workers to the pheromonal emission source. Therefore, it is likely that the distinctive natural products produced by both the queen and worker honey bee have been evolved to subserve functions that are related to the specific roles that the sting plays as a defensive organ for members of each caste.

The esters produced on the sting apparatus of the queen honey bee are novel insect natural products that are primarily based on decanoic acid as the acidic moiety of the esters. Significantly, honey bees have produced a variety of other compounds that are based on C_{10} acids. For example, queens produce 9-hydroxy-(*E*)-2-decenoic acid in their mandibular glands (Butler *et al.*, 1964) and this compound is accompanied by several other C_{10} acids (Callow *et al.*, 1964; Pain *et al.*, 1962). Workers also produce a distinctive decenoic acid in their mandibular glands, 10-hydroxy-(*E*)-2-decenoic acid (Butenandt & Rembold, 1957), demonstrating that the biosynthesis of C_{10} compounds is not limited to queens. Both castes of the honey bee have evolved pathways in which C_{10} compounds are emphasized. Although the biochemical significance of synthesizing a variety of natural products based on C_{10} acids is unclear, the production of these novel exocrine constituents documents them as very characteristic products of these social insects.

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