

OXYGENATED COMPOUNDS IN BEESWAX: IDENTIFICATION AND POSSIBLE SIGNIFICANCE

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Abstract—1. Beeswax synthesized by non-foraging honeybee workers contains six oxygenated volatiles in addition to a series of normal alkanes.

2. Decanal constitutes nearly 50% of the oxygenated volatiles and is accompanied by octanal, nonanal, furfural, benzaldehyde and 1-decanol.

3. The possible significance of the aldehydes as stimulators of hoarding behaviour and attractants for wax moths is discussed.

INTRODUCTION

Beeswax, an important structural material of honeybees, is produced as wax scales by worker abdominal glands and is utilized for the formation of comb. Nearly 200 yr ago Hüber (1792) concluded that the consistency of comb was appreciably different from its original wax scale state after manipulation by the workers' mouthparts with the presumed addition of glandular constituents. Therefore, the material that constitutes comb wax is presumably derived from both abdominal and cephalic, and possibly, thoracic secretions. Furthermore, its provenance notwithstanding, it is known to be an extremely complex product containing a large number of aliphatic constituents.

Typically, comb beeswax is characterized by the presence of long-chain hydrocarbons, acids, alcohols and primarily esters (Warth, 1956). On the other hand, the characteristic aroma of beeswax is obviously not identified with these relatively non-volatile constituents, and a number of low- and medium-boiling compounds have been identified from wax cappings. Ferber and Nursten (1977) conclusively identified nearly 30 compounds in beeswax and another 28 tentatively. However, many of these compounds are typical plant natural products and probably represent volatiles originally derived from pollen and/or nectar which had been subsequently absorbed by the beeswax. In short, there is no information available on the identities of the low-boiling exocrine compounds derived from bee workers that are present in virgin comb and are responsible for its strong aroma, as first noted by Hüber (1792).

In the present paper, we report on the identification of very volatile oxygenated compounds that are present in virgin comb constructed by worker honeybees not permitted to forage and collect plant products. In addition, we have attempted to determine if the identified volatiles would increase the hoarding of sugar syrup in laboratory cages, since Rinderer

(1981) demonstrated that volatiles from empty comb increase sugar syrup hoarding in laboratory cages.

MATERIALS AND METHODS

Beeswax collection

In Louisiana, virgin comb was obtained from two honeybee colonies with open-mated queens of commercial stock. The colonies were shaken to form artificial swarms with caged queens and fed, *ad libitum*, 50% (w/w) sucrose solution. Newly made comb, uncontaminated by brood rearing or food storage, was harvested for chemical studies.

Chemical analysis

Comb was steam distilled and the concentrated volatiles extracted with diethyl ether in a closed system. The ether extract was concentrated under nitrogen and analyzed directly on an LKB-9000 gas chromatograph mass-spectrometer utilizing a 3.6 m × 2 mm i.d. glass column packed with 3% OV-17 programmed from 50 to 300°C at 8°C/min.

Bioassays

Five different compounds and an equal parts by volume mixture of these compounds were bioassayed for their effect on sugar syrup hoarding rate: octanal, nonanal, decanal, 1-decanol and pentadecane. The compounds were applied to paper wicks and reapplied daily to deliver an evaporated dose of 0.006 mol/day for the low rate and 0.024 mol/day for the high rate. The wicks were in an acrylic plastic treatment chamber through which air was pumped by an air pump. The chamber did not contain any compounds for the controls, which were otherwise identical.

Recently emerged worker bees less than 24 hr old were placed in groups of 30 per hoarding cage and placed in incubators at 35°C, 50% relative humidity. Each replicate group of four cages from one source colony was placed in a large polyethylene bag which was tied closed, but not sealed. Three groups from one colony were placed on separate shelves in each of eight incubators, with one treatment randomly assigned to each shelf. Each incubator had an aquarium air pump pumping air through a separate chamber for each treatment. The treated air entered the bags and was then divided between four tubes, one entering each

replicate cage. An exhaust fan pulled air from the incubators and vented it outside so that air pumped into the treatment chamber was not recycled and contaminated with the tested compound(s).

Each compound and the mix were tested for one randomly chosen week, using eight source colonies per week. Some of the source colonies were used more than once, if brood was available.

The effect of the compounds was measured as changes in sugar syrup hoarding rate, as previously measured by Rinderer (1981) for comb volatiles.

RESULTS

Six oxygenated volatiles were identified in the steam distillate of virgin comb wax. Identifications were based on their congruent mass spectra with standard compounds (Stenhagen *et al.*, 1969) in combination with their identical retention times with the standards. The average percentage of each compound (six replicates) is presented in parentheses after the name of the volatile.

The major compound identified was decanal (46%), and this aldehyde was accompanied by its corresponding carbinol, 1-decanol (10%). Nonanal (18%) was the second most quantitatively important aldehyde present, eluting shortly after octanal (6%), the lowest boiling aliphatic carbonyl compound detected. In addition, one heterocyclic aldehyde, furfural (10%), was detected along with an aromatic constituent, benzaldehyde (10%).

A series of normal hydrocarbons was also present in the steam distillate. The lowest boiling compound detected was nonane; C₁₅-C₂₇ alkanes were readily identified as concomitants.

Certain of these compounds seemed to increase or decrease hoarding rates at some level but none of these changes were statistically significant. Generally, all of the compounds significantly and substantially increased the variance of the hoarding responses. Under treatment, some colonies increased hoarding dramatically while the same treatment caused some colonies to nearly cease hoarding.

DISCUSSION

The bouquet of freshly constructed comb is clearly dominated by aldehydes, with three straight-chain compounds—octanal, nonanal and decanal—constituting about 70% of the mixture. These three aldehydes, as well as benzaldehyde, were previously identified from cappings of beeswax by vacuum distillation, along with about 45 other compounds (Ferber and Nursten, 1977), many of which are almost certainly derived from plant sources, possibly nectar and pollen, and even propolis. On the other hand, neither furfural nor 1-decanol have been previously identified as constituents of beeswax. Indeed, furfural has not previously been detected as an insect natural product, in contrast to four of the other oxygenated volatiles which have been characterized as defensive allomones of a variety of insect species (Blum, 1981). By contrast, the major carbonyl compound present, decanal, does not appear to have been identified as an exocrine product of any arthropod species.

The quantitative dominance of decanal further emphasizes the biosynthetic predilection of honey-

bees for normal C₁₀ compounds. This point was previously emphasized when it was determined that normal C₁₀ alcohols and acids were characteristic of the esters present on the sting shaft of the queen bee (Blum *et al.*, 1983). Since C₁₀ compounds constitute important pheromones of the queen bee (Butler *et al.*, 1961) and C₁₀ products dominate the mandibular gland secretion of young workers (Butenandt and Rembold, 1957), and are also important sting alarm pheromones (Blum *et al.*, 1978), the identification of another 10 carbon compound, decanal—and 1-decanol as well—is not necessarily surprising. On the other hand, the *raison d'être* of the biosynthetic virtuosity of the honeybee on a normal C₁₀ theme still remains *terra incognita*.

The identified aldehydes would seem to constitute ideal candidates as the stimulators of the hoarding behaviour observed by Rinderer and Baxter (1978, 1979) with empty honey storage-comb surface area, and Rinderer (1981) with volatiles from empty comb. Although the observed results with three aldehydes and 1-decanol are consistent with the hypothesis that volatiles from empty comb regulate the intensity of hoarding (Rinderer, 1981), they do not provide a definitive evaluation of the compounds bioassayed.

Although the function of these aldehydic constituents remains to be unambiguously established, it is possible that they possess an unsuspected role that would not be at all adaptive for honeybees. Females of one of the major pests of honeybee colonies, the wax moth, *Galleria mellonella*, are attracted to a male-derived sex pheromone that consists primarily of nonanal with undecanal constituting a quantitatively less significant pheromone (Leyrer and Monroe, 1973). Both nonanal and undecanal are attractive to females with the former compound being far more active. It is not unlikely that female wax moths may be attracted by the nonanal in the comb of honeybees, and possible octanal and decanal as well. These moths, which are very sensitive to their aldehydic sex pheromones, would thus be attracted to the rich wax resource of their bee hosts, because it possesses an aroma for which the moths have evolved a great olfactory acuity.

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