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CHEMISTRY OF THE STING APPARATUS OF THE WORKER HONEYBEE *

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Summary

Volatile compounds present in extracts of the sting apparatus of foraging worker honeybees were analysed by gas chromatography and mass spectrometry. Eight acetates were detected, including n-butyl, isoamyl, n-hexyl, n-octyl, n-decyl, and benzyl acetate. These esters were accompanied by isoamyl alcohol, 2-nonanol and benzyl alcohol, as well as a series of aliphatic hydrocarbons

Introduction

The chemical basis of alarm behaviour in the honeybee (*Apis mellifera*) has been primarily identified with isoamyl acetate, a compound demonstrated to be present on the worker sting apparatus (Boch, Shearer & Stone, 1962). However, the presence of additional chemical releasers of alarm behaviour was indicated by the fact that guard bees would attack and sting a cotton ball to which freshly excised stings had been added, but not one treated with isoamyl acetate alone; Free and Simpson (1968) reached a similar conclusion. Boch, Shearer and Petrasovits (1970) demonstrated that a sting was significantly more active than isoamyl acetate in alerting and attracting bees at the hive entrance.

Gas-chromatographic analyses of extracts of honeybee stings have shown that the sting apparatus is a rich source of both low- and high-boiling compounds (Boch & Shearer, 1966; Gunnison & Morse, 1968). As part of a programme for studying the chemical bases of defensive behaviour in honeybees, we have analysed the volatile compounds present in extracts of the sting apparatus of worker honeybees.

Materials and Methods

Stings were collected from workers that originated from three colonies in Baton Rouge, LA, USA, which were considered to be of average temperament, neither too aggressive nor too gentle. Workers were collected in plastic bags which were transferred to a refrigerator at 7°C. After the bees were immobilized, the sting apparatus (100/vial), together with the motor mechanism and attached glands, was pulled from the bee's body with fine forceps. Included with the excised sting apparatus was the setose lobe which enfolds the base of the sting shaft in which the volatiles are located (Ghent & Gary, 1962). The sting apparatuses were transferred to reaction vials (100/vial), containing 70 mg of anhydrous sodium sulphate and 0·5 ml of reagent methylene chloride, which were nested in dry ice. The vials were wrapped in Parafilm and stored at −8°C before being shipped to Athens, GA, and Bethesda, MD, for analyses.

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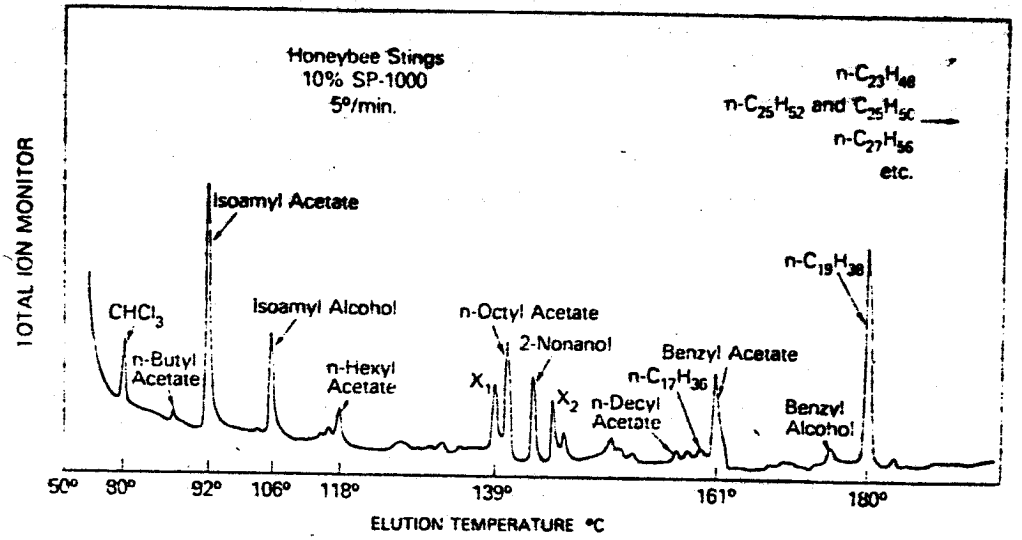

FIG. 1. Gas chromatogram of an extract of worker honeybee stings. X_1 and X_2 correspond to unidentified acetate esters.

TABLE 1. Oxygenated compounds in extracts of the worker honeybee sting apparatus.

Compound	*Significant mass spectral ions (m/e)*	*Mean percentage ± SE*
n-butyl acetate	43*b*, 56, 61, 73, 116*a*	1±0·3
isoamyl acetate	43*b*, 55, 70, 130*a*	27±6·2
isoamyl alcohol	43, 55*b*, 56, 57, 70, 88*a*	12±3·7
n-hexyl acetate	43*b*, 55, 56, 61, 69, 84, 144*a*	3±0·7
X_1 (acetate ester)	43*b*, 55, 56, 57, 61, 69, 70, 71, 83, 87, 98, 99, 112, 126, 142, 143	9±2·8
n-octyl acetate	43*b*, 55, 56, 57, 70, 172*a*	14±4·1
2-nonanol	43, 45*b*, 55, 69, 144*a*	9±3·2
X_2 (acetate ester)	43*b*, 54, 55, 57, 67, 68, 69, 81, 82, 95, 110, 127, 128	6±2·1
n-decyl acetate	68, 69, 70*b*, 71, 82, 83, 84, 94, 112, 140, 200*a*	1±0·5
benzyl acetate	43, 79, 90, 91, 108*b*, 150*a*	13±4·5
benzyl alcohol	50, 51, 77, 79*b*, 107, 108*a*	3±1·1

a molecular ion *b* base peak

Extracts (6 replicates) were analysed without further treatment on a LKB-9000 gas chromatograph—mass spectrometer using a 3·66-m column of 10% SP-1000 as a stationary phase. The column temperature was programmed from 60 to 200°C at 5°/min. Peak areas were determined by electronic integration.

Results

The complex mixture of components in extracts of the sting apparatus is fairly well resolved by gas chromatography (GC). All identified compounds that eluted up to 180°C are indicated in Fig. 1, and a series of high-boiling hydrocarbons that eluted above this temperature are noted in the upper right hand corner. Identifications of the oxygenated compounds were based on congruent mass spectra and GC retention times with standard compounds. These compounds and their significant mass spectral characteristics are listed in Table 1, together with the mean percentage of each compound, based on the average from 6 chromatograms.

Isoamyl acetate has been previously identified by Boch, Shearer and Stone (1962) as the major low-boiling compound present in extracts of the honeybee sting apparatus. It is accompanied by eight other acetates (Fig. 1), two of which have not been chemically identified. The presence of three alcohols in these extracts demonstrates that this class of compounds is also a qualitatively important feature of the honeybee sting apparatus.

A large number of normal alkanes and alkenes were also identified. The main hydrocarbon was nonadecene (Fig. 1); n-heptadecane, n-heneicosane, n-tricosane, n-pentacosane and n-heptacosane accompanied the C_{19} alkene. The C_{17}–C_{27} alkenes corresponding to the saturated hydrocarbons were also present.

The amounts of the oxygen-containing compounds in the extracts demonstrate that isoamyl acetate, isoamyl alcohol, n-octyl acetate and benzyl acetate constituted about two-thirds of the lower-boiling volatiles (Table 1). All extracts analysed showed n-butyl acetate, n-hexyl acetate, n-decyl acetate and benzyl alcohol as minor components; compounds X_1, 2-nonanol and X_2 accounted for about one-fourth of the oxygenated volatiles in all extracts (Table 1). The percentage values obtained for these compounds changed little even after prolonged storage of the extracts at 4°C.

Discussion

The low-boiling constituents associated with the sting apparatus are dominated by acetate esters, which account for about 60% of this fraction. As previously demonstrated by Boch, Shearer and Stone (1962), isoamyl acetate is the major ester present, but its corresponding free alcohol is also a quantitatively important constituent. The presence of at least 8 acetates, including at least one aromatic ester (benzyl acetate), demonstrates that the sting apparatus is an especially rich source of these volatile compounds. Two of the three alcoholic concomitants of these acetates—isoamyl alcohol and benzyl alcohol—may be biosynthetically related to their corresponding esters, both of which are present in considerably greater quantities than the alcohols. On the other hand, 2-nonanol is distinctive because it is not accompanied by its acetate ester, and because it is the only secondary alcohol detected as a sting volatile.

It seems remarkable that isoamyl alcohol and acetate should replace so completely the expected n-amyl group in the series. This suggests an important crossing of short-chain fatty acid and mevalonate pathways in worker honeybees.

Several of these oxygenated compounds have been detected previously as exocrine products of insects: 2-nonanol has been identified as a mandibular gland product of several *Trigona* species (Luby et al., 1973; Blum, 1974); where it appears to function as both part of a trail pheromone and a defensive secretion. Isoamyl alcohol has been detected as a trace constituent in the defensive secretion of a gyrinid beetle (Schildknecht et al., 1972), whereas benzyl alcohol has been identified in the presumed aphrodisiacal secretions of male noctuid moths (Aplin & Birch, 1970). n-Hexyl acetate appears to be a typical defensive product of adult coreids (Waterhouse & Gilby, 1964), whereas n-octyl acetate has rarely been detected as a hemipterous defensive compound (Baggini et al., 1966). n-Decyl acetate appears to be a characteristic product of Dufour's gland in formicine ants (Bergström & Löfqvist, 1968), and probably functions as an alarm pheromone.

The alkanes and alkenes that were identified in extracts of the sting apparatus may have more than a structural function. It is possible that they reduce the volatility of the lower-boiling compounds, as first suggested by Gunnison and Morse (1968). This suggestion should be amenable to easy testing.

Boch and Shearer (1971) have evaluated a large series of esters, ketones, aldehydes and alcohols as releasers of alarm behaviour for honeybees. Isoamyl acetate was the most

active ester tested, although n-butyl acetate was also reported to be quite effective as a chemical releaser of alarm. Neither n-hexyl acetate nor n-octyl acetate was reported to be a very active alarm pheromone, and benzyl acetate was not active at all. In our laboratory, evaluation of each oxygenated compound in a bioassay, using caged bees, demonstrated that all compounds elicited some level of response as releasers of alarm behaviour (A. M. Collins, unpublished).

Since honeybee workers are exposed to a mixture of sting volatiles, not to a single compound, caution should be exercised in drawing conclusions about the alarm-releasing activities of the individual volatiles. Evaluation of mixtures of these compounds, at concentrations similar to those in the extracts, will be required in any attempt to comprehend the significance of the rich blend of volatiles produced in the sting apparatus. This subject forms the basis for continued research in our laboratories.

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