2-Heptanone in the Mandibular Gland Secretion of the Honey-bee

WORKER honey-bees produce alarm pheromones in their sting apparatus and in their mandibular glands[1]. One of the components of the sting pheromone[2] has been identified as isoamyl acetate which releases strong alarm behaviour in bees. When filter paper treated with isoamyl acetate is placed at the hive entrance the bees nearby become alerted and greatly agitated. They assume a characteristic aggressive posture and run jerkily in circles or short zigzags preferentially toward the paper. The same behaviour can be observed when mandibular glands or crushed heads of worker bees are presented at the hive entrance.

The secretion contained in the mandibular gland reservoir has a strong smell which, according to Forel[3], is quite similar to that of the secretion of the anal glands of certain ants (for example, *Tapinoma erraticum*, Latr.).

We have identified an alarm substance from the mandibular glands of the honey-bee as 2-heptanone.

Heads of bees were collected and held at dry-ice temperature until used. Pentane or petroleum ether (0·5 ml.) was added to 10 heads which were then crushed with a flat-ended spatula and mixed thoroughly with the solvent. After centrifuging, the supernatant liquid was drawn off and a 5-μl. aliquot was injected into a MicroTek *GC2500R* gas chromatograph equipped with a hydrogen flame ionization detector. In the lower molecular size range a single prominent peak was present. A typical ketonic odour was readily detectable at the instrument outlet when this component emerged. The retention times on three different columns are compared in Table 1 with those for an authentic sample of 2-heptanone (purchased from Eastman Organic Chemicals, Rochester 3, New York).

Table 1. GAS CHROMATOGRAPHY OF BEE-HEAD EXTRACTS AT 70° C

Column	Column length (ft.)	Retention times (min) Bee head component	2-Heptanone	Reduced bee head component	2-Heptanol
10% 'Apiezon *L*'	6·5	6·25	6·25	7·78	7·72
2% 'Carbowax *M*20'	8	7·25	7·2	17·4	17·1
10% '*DEGS*'	4·5	2·50	2·50	3·9	3·8

Column support: 'Anakrom *ABS*' 150/160 mesh. Carrier gas: nitrogen, 70 ml./min.

A portion of the bee-head extract was treated in ether with sodium borohydride. The resulting alcohol was also subjected to gas chromatography and the retention times (Table 1) are compared with a sample of 2-heptanol.

From an extract of 200 heads of forage bees a fraction was separated by gas chromatography and collected[4] for analysis by infra-red spectrometry. The spectrum of the isolated component was identical to that of authentic 2-heptanone. A 2,4-dinitrophenylhydrazone derivative was prepared, but insufficient material was obtained for a determination of the melting point. However, thin layer chromatography of the derivative on a 'Kieselgel *G*' plate (chloroform developed) showed the same R_F as a sample of the 2,4-dinitrophenylhydrazone of 2-heptanone.

In order to show that 2-heptanone did originate in the mandibular glands, the glands were excised from the heads of five bees and were analysed after extraction with 0·1 ml. petroleum ether. The yield was 8·6 μg of 2-heptanone per bee. This represents about 40 per cent of the amount recovered from the crushed heads of foraging bees, which is in the range of 15–23 μg per bee. The difference is probably due to loss during dissection. Heptanone was not recovered from bees which had not yet flown, nor from the heads of either young or old queens, nor from the heads of drones.

Hexane extracts of heads of foraging bees and a solution of authentic 2-heptanone in paraffin oil were tested for their effect on honey-bees. The substances were applied to small corks which were set one at a time on the alighting board directly in front of the entrance to the hive. Guard bees became alerted and agitated. These bees came forth from the hive and attacked the cork and removed it from the hive entrance. Control corks with paraffin oil alone did not elicit any of these reactions.

This is the first reported occurrence of a heptanone in bees. However, in the dolichoderine ant, *Iridomyrmex pruinosus*, Roger, 2-heptanone has been identified as a constituent of its alarm substance[5].

D. A. SHEARER
Analytical Chemistry Research Service,
R. BOCH
Entomology Research Institute,
Canada Department of Agriculture,
Ottawa.

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MICROBIOLOGY

Nucleotide Composition of Soluble Ribonucleic Acid of *Streptomyces fradiae*

THE nucleotide composition of bacterial DNA varies widely from species to species and the $G + C$ moles per cent particularly range from 30 to 70. Instead, the $G + C$ moles per cent of *s*RNA seem to remain at about 60–62 per cent in almost all species examined. A few results were available for species with high $G + C$ DNA content; thus, in order to explore this range in other bacterial species, we have examined the nucleotide composition of the *s*RNA of *Streptomyces fradiae PSA* 156 (DNA $G + C$ content of this strain is 72·4 from buoyant density and 78·5 from temperature of denaturation midpoint determination)[1].

The cells were grown at 30° while shaking in a medium containing 0·5 per cent 'Bactopeptone', 0·3 per cent yeast extract, 0·2 per cent beef extract, 0·1 per cent casamino-acids and 0·1 per cent glucose. After several hours, the cells were collected and washed in 0·01 M *tris* buffer (*p*H 7·3) containing 0·01 magnesium acetate.

*s*RNA was prepared according to the method of Tissières[2] from the bacterial extract from which the ribosomal fraction had been carefully removed (180 min at 105,000*g*). Ultracentrifugal analyses showed that the sedimentation coefficient of purified *s*RNA was 4*S*.

Spectrophotometrical analyses revealed the characteristic absorption spectrum of RNA.

*s*RNA was precipitated by cold 0·5 M PCA, subjected to 0·3 M KOH hydrolysis for 18 h at 37° C, then adjusted to *p*H 1 and *p*H 3·5 with cold PCA[3]. The nucleotides were fractionated by paper electrophoresis in ammonium formate buffer *p*H 3·5 at 1,000 V, 4 m.amp, for 5 h 30 min[4]. On the electrophoresis paper the spots were clear and separated. The guanilic acid spot extended dividing two components, both of which showed the same characteristic absorption spectrum of guanilic acid. Following the method of Davidson and Smellie, we retained these two components isomeric guanilic acids and properly guanilic 2′ and 3′ phosphate. In the result the value of guanilic acid comprised both isomeric compounds.

The separated bands were eluted in *p*H 2 1 N formic acid and ultra-violet absorbancy of the eluate was determined.

The results were expressed as moles of each component, per cent moles of all nucleotides.

The analyses of nucleotide composition showed: adenylic acid, 19·5 per cent; cytydilic acid, 26·8 per cent; guanilic acid, 35·2 per cent; and uridylic acid, 18·5 per cent.

The standard deviation for the mean values of each component was in the range of 1·2–2·5 per cent.