

# P188 Royal jelly of *Apis cerana japonica* acts as a bee-attracting pheromone

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## Introduction

The flower of the oriental orchid *Cymbidium floribundum* is known to attract the Japanese honeybee *Apis cerana japonica* not only workers but also drones and queen, that is, to attract even swarm and absconding-bees.



A mixture of 3-hydroxyoctanoic acid (**3-HOAA**) and 10-hydroxy-(*E*)-2-decenoic acid (**10-HDA**) was identified as the active principles from the orchid flower.

{*C. Floribundum* Attracts the Japanese Honeybee with a Mixture of 3-HOAA and 10-HDA, Michio Sugahara, et al.(2013) Zool. Science Vol.30(2)}

Both compounds are also mandibular gland components of worker honeybees (*A. cerana japonica*) with related compounds. In this study, aliphatic compounds in the mandibular gland of nurse and forager workers were analyzed by GC-MS. Aliphatic compounds in royal jelly made in the mandibular gland were also analyzed. In addition, by a bioassay, a queen cell containing royal jelly was demonstrated to attract bees and the royal jelly constituents which have attraction activity were identified. Finally, it was shown that royal jelly acted as a bee-attracting pheromone.

## Materials and Methods

The Japanese honeybee reared in Moriguchi, Osaka Pref., were used for bioassay.

Gas chromatography-mass spectrometry (GC-MS) analysis was conducted using a Network GC System (6890 N; Agilent Technologies Inc.) coupled with a mass selective detector (5975 Inert XL; Agilent Technologies Inc.)

Seven of the compounds quantified were obtained commercially.

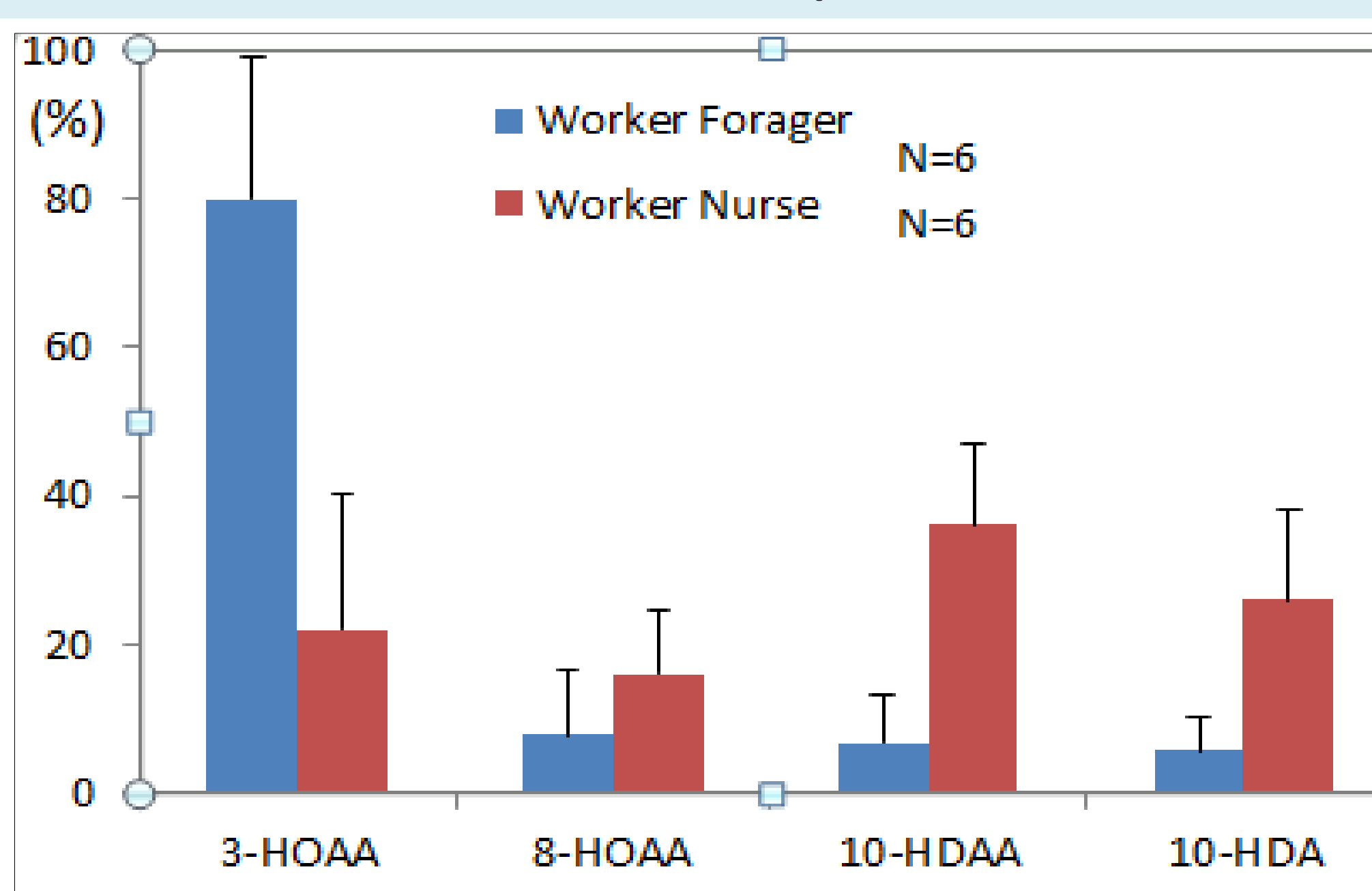
Tissue connected to the mouthpart was removed from a bee with tweezers under a stereomicroscope. Diethyl ether was added for extraction.

Royal jelly was scraped out from the queen cell with a spatula. Diethyl ether was added for extraction. Salt precipitation was performed.

## Results

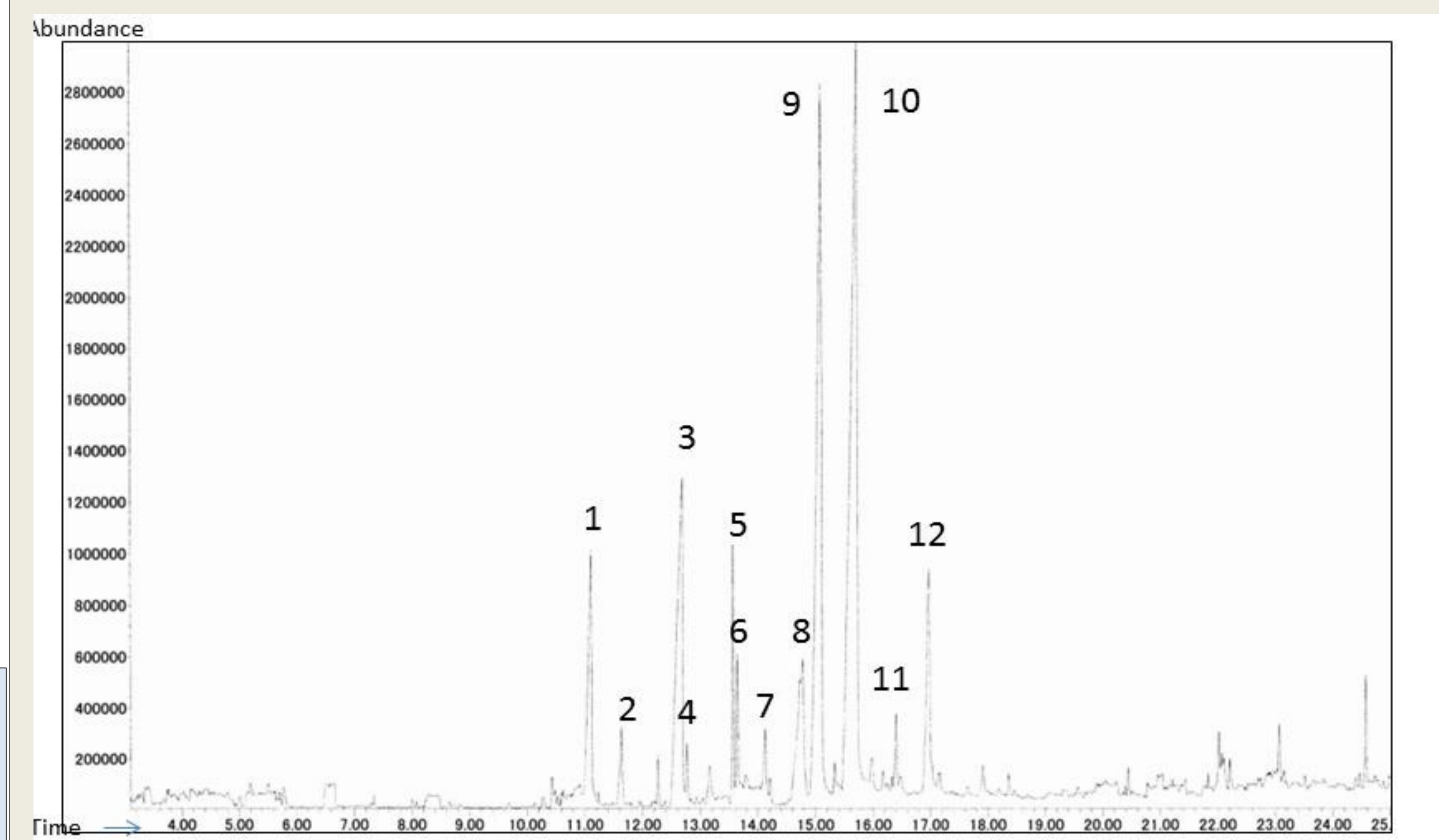
### GC-MS analysis of the mandibular gland

The amount of 3-HOAA differed greatly between the nurse and forager. In the forager, the amount of 3-HOAA was large and that of other 3 compounds was small. In the nurse, the amount of the 4 compounds was almost equal.



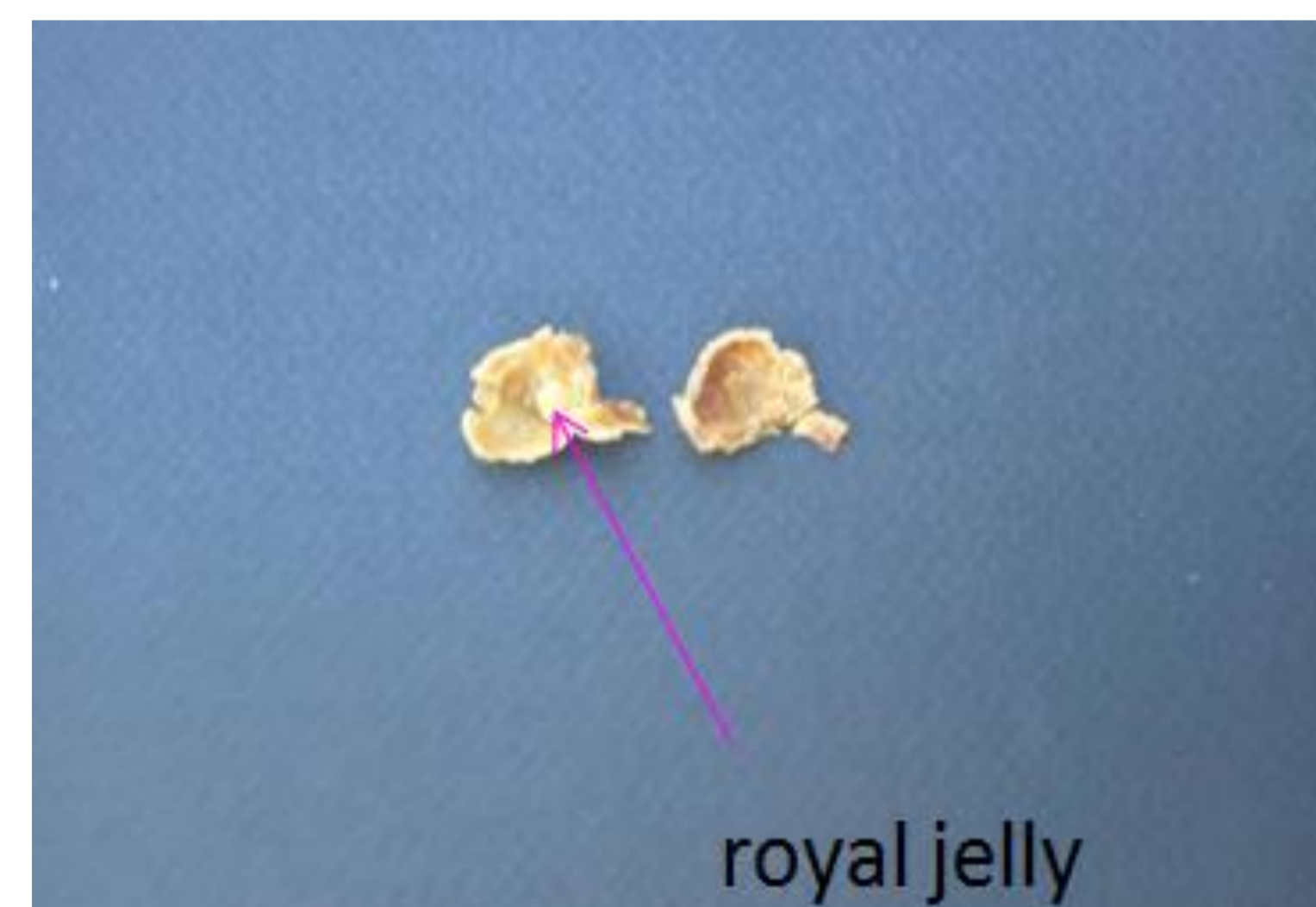
## GC-MS analysis of royal jelly

A typical GC-MS chromatogram is shown in Fig.

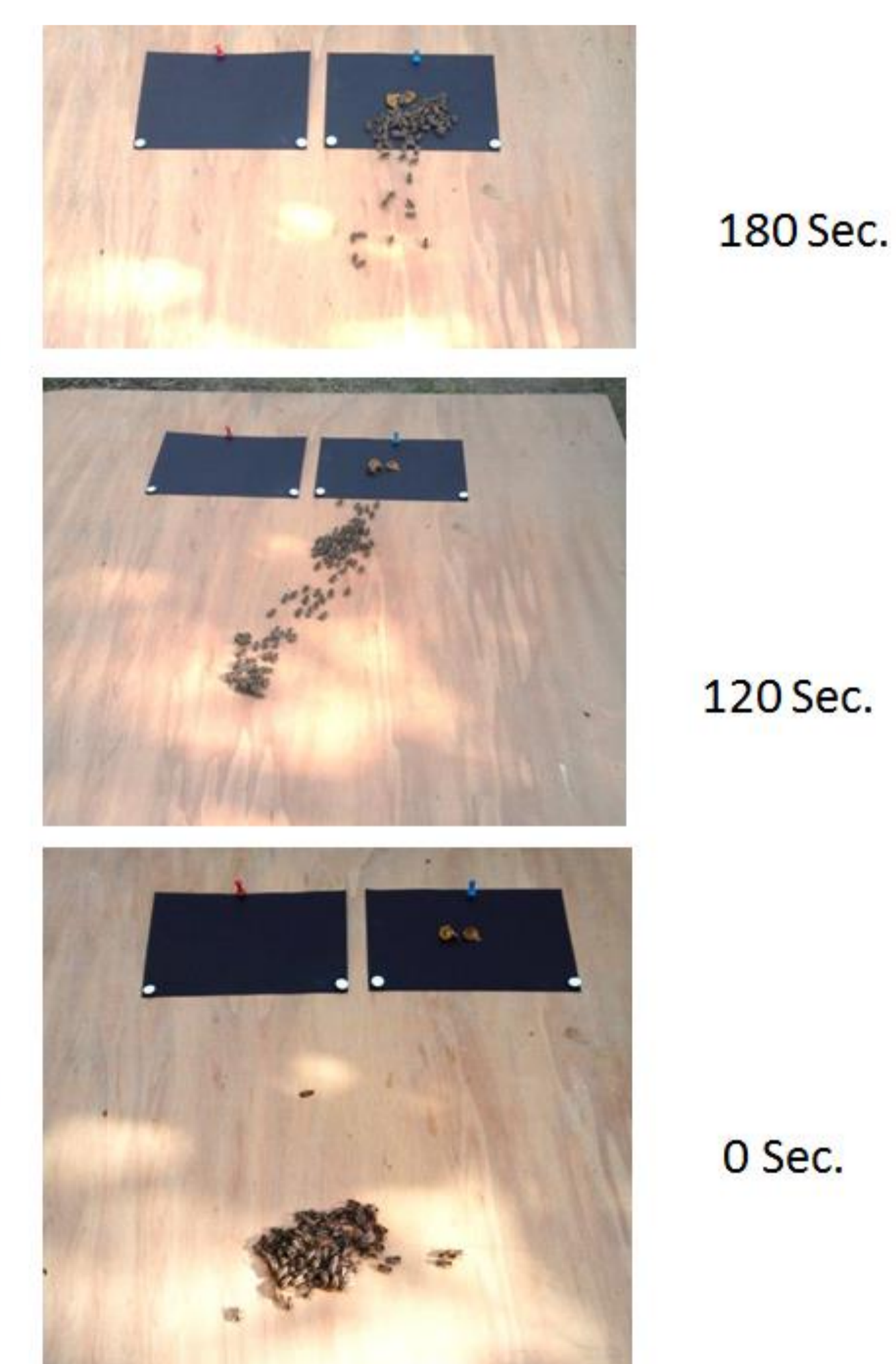


Peaks; **1, 3-HOAA**, 2, unknown 3, 8-HOAA, 4, HOB, 5, BHT(2,6-bis(1,1-dimethylthyl)-4-methylphenol (stabilizer of diethyl ether), 6, 3-hydroxydecanoic acid, 7, 9-hydroxydecanoic acid, 8, 9-HDA, 9, 10-HDAA, **10, 10-HDA**, 11, unknown, 12, unknown. Peak 6, 7 was identified based on the MS database (Wiley275 library; Hewlett Packard Co.). Other peaks were identified based on GC-MS analysis of standard reagents.

## Activity of a queen cell (royal jelly) in bioassay



A larva was removed from the queen cell, the queen cell was dissected to expose royal jelly, and bee gathering behavior to the queen cell was observed. The result is shown in Fig. More than half of the bees dropped on the plywood board gathered to the queen cell.



## Discussion

Our assay results showed that royal jelly attracted bees as the flower of the oriental orchid did.

The attracting effect was found to be caused by 3-HOAA, 10-HDA in the royal jelly. As shown in Fig, bees surround a queen cell queen in the same way as they surround a queen. This phenomenon is thought to be caused by attraction effect of 3-HOAA, 10-HDA in a queen cell.

In our analysis, the amount of 3-HOAA in royal jelly was higher compared with the composition of royal jelly of *A. mellifera* reported by Isidorov et al. (2009).

