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CHEMISTRY OF SEX PHEROMONE EXTRACTED FROM THE AMERICAN COCKROACH, *Periplaneta americana* L.: AN INNOVATION IN HOUSE HOLD PEST MANAGEMENT

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ABSTRACT

Cockroaches are one of the most adaptable and successful insect (pest) groups to ever inhabit this planet. American cockroach, *Periplaneta americana*, has a wide geographic distribution and as is one of the few household insect species known. Pheromone control of cockroach would be a promising approach in the modern era. Accordingly experiments have been designed and executed. The GC analysis of the pheromones extracted from the cuticular abdominal glands of female American cockroach contained nearly more than twenty compounds, of which around seven compounds were found to be abundant in the extract. The fraction identified at 19th minute was observed to be 100% abundance. This part of analysis revealed that the cuticular abdominal gland of American cockroach contained 2-Butanone as pheromone source. Further many more peaks were noted in the spectrum that suggested that the pheromone compound is not a single compound rather mixture of compounds or associated with their intermediate metabolites with the functional groups like aldehydes, alcohols and acids of both alkenes and alkanes. The MS data clearly spell out that the sex pheromones of cuticular origin in American cockroach is a blend of both aliphatic and aromatic compounds. Mostly the chemical nature of the sex pheromone was reported as aromatic compound with several atoms are protruded on the outer surface of the molecule. These atoms were suspected to have affinity towards the transmembrane protein of the male antennae and their sensory cells.

Keywords: *Periplaneta americana*, Sex pheromone, GC MS analysis, Pest management.

INTRODUCTION

Insects are reported as analytical chemists *par excellence*. They perceive the world through small molecules which carry information (signature) for the recognition of potential mates, prey, and specific features of the environment, such as food sources, oviposition sites, etc. These information-carrying chemical compounds are referred as *semiochemicals.* Semiochemicals are subdivided into allelochemicals and pheromones, depending on whether the interactions are interspecific or intraspecific. Intraspecific communications such as, sex attractants have been labeled as "Pheromones" [1] Cockroaches are one of the most adaptable and successful insect (pest) groups to ever inhabit this planet. Generally there are six species of cockroaches that can become pests: German cockroach, brown banded cockroach, oriental cockroach, smoky brown cockroach, American cockroach, and Turkestan cockroach. American cockroach, *Periplaneta americana*, has a wide geographic distribution throughout much of Asia and particularly in developing countries like India, China, and as is one of the few household insect species known [2]. A sex pheromone is produced in an adult female-specific cuticular abdominal gland located on the anterior of the last abdominal tergite in the female cockroach [3]. Pheromone control of cockroach would be a promising approach in the modern era.

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MATERIAL AND METHODS

Preparation of Pheromone Extracts

Cuticular abdominal glands were excised with a pair of fine scissors from virgin female cockroach. The tissue was extracted in hexane for 15 min. at room temperature. Extracts were placed in screw cap vials and stored at −20°C until analysis. In addition, the extracted chemical moieties were further subjected to purification with 2 ml of hexane for 10 min. followed by two extractions with 1.5 ml for 1 min each and the extracts were pooled together. Lipids were separated by TLC in hexane/diethyl ether/formic acid (80:20:2) or hexane/diethyl ether (90:10).For some experiments, specific compounds were extracted in diethyl ether or Tricholoro methane or methanol or water or acetic acid. The identified chemicals moieties were correspondingly compared with standard compounds purchased from Sigma (India). The GC peak values of standard were compared with GC spectrum of our sample.

Gas Chromatography

GC analysis was conducted by using a DB-23 column(30 m×0.25 mm Internal Diameter (ID), J&W Scientific, Folsom, CA, USA) and an HP-5MS (25 m×0.25 mm ID, Yokokawa Hewlett-Packard, Tokyo, Japan) column with a Shimadzu GC-17A gas chromatograph equipped with a flame ionization detector (FID) and a split less injector system. Helium was used as the carrier gas. The DB-23 column temperature was set initially at 100°C for 2 min. and then increased to 250°C at 5°C min^{-1}; the temperature of the detector and injector was 250°C. The HP-5MS column temperature was initially set at 150°C for 2 min. and then increased to 260°C at 10°C min^{-1}.

Fourier Transfer Infrared Spectroscopy (FT-IR) Analysis

Working principle

The application of traditional infrared spectroscopy for low concentration measurements, such as ambient and air measurements is limited by several factors. First is the significant presence of water vapour, $CO_2$and methane, which strongly absorb in many regions of the infrared (IR) spectrum. Consequently, the spectral regions that can easily be used to search for pheromones are limited to 760-1300cm -1, 2000-2230 cm -1, and 2390-3000cm -1. Another problem is that the sensitivity is not enough to detect very small concentrations in the sub-ppm level. Finally, spectral analysis was difficult, since subtraction of background spectra had to be carried out manually. The development of Fourier Transform Infra Red spectroscopy (FTIR) in the early1970s provided a quantum leap in infrared analytical capabilities for monitoring trace amount of pheromones in ambient air. This technique offered a number of advantages over conventional infrared systems, including sensitivity, speed and improved data processing.

The IR source used in the Temet GASMET FTIR CR-series is a SiC ceramic at a temperature of 1550 K. The IR radiation goes through an interferometer that modulates the infrared radiation. The interferometer performs an optical inverse Fourier transform on the entering IR radiation. The modulated IR beam passes through the gas Sample, where it is absorbed to various extents at different wavelengths by the various molecules present. Finally, the intensity of the IR beam is detected by a detector, which is a liquid-nitrogen cooled MCT (Mercury-Cadmium-Telluride) detector in the case of the Temet GASMET FTIR CR-series.

Gas Chromatography-Mass spectrometry (GCMS)

Gas Chromatography-Mass Spectrometry (GC-MS) analyses were conducted with a Hewlett-Packard 5972 MSD coupled to a Hewlett-Packard 5890 series II gas chromatograph equipped with split less injection and an HP-5MS column. The column oven temperature was held at 100°C for 2 min. and then increased to 240°C at a rate of 10°C min^{-1}. The GC-MS analyses were done in a Shimatzu QP5000 instrument under computer control at 70 eV. Chemical ionization was performed using ammonia as reagent gas at 95 eV [4]. The identified compounds were then compared with standards run under the same conditions. The obtained spectral data were already stored in a compact library of chemical substances (NIST62.LIB). As mentioned earlier, fresh samples were fractionated and collected in separate storage glass vials for each fraction until further use. The volatiles from the distilled fractions were subjected to GC for crosschecking and confirmation of compounds in each fraction.

RESULTS

The data depicted in Gas Chromatograph revealed that the cuticular abdominal gland contained 20 to 25 chemical constituents of which, seven compounds were found to be more abundant than that of the other minor fractions.(Fig.1). Further studies were extended to find out the functional groups of the pheromone extracted from the cuticular abdominal glands of female cockroach. The data given in the IR spectrum clearly proved that the glandular extract contained several chemical compounds with aldehydes, keto groups, alcohol, acids of both aliphatic and aromatic compounds (Fig.2). The subsequent Mass spectral analysis further authenticated the mass value of each identified compound based on the comparative analysis with the standard compound spectral values (available in equipment - CHEM-LIB database), the percentage of similarity between the identified compound and standard were evaluated and determined the name of the compounds (Fig.3).

The documents related to the study of perceptual mechanisms of pheromonal cueing in relationship to

complex sexual behaviors has been hampered by the lack of identification of specific compounds functioning as conspecific chemical messengers. Hence, the present study reported the chemical nature of the pheromones present in female cockroach. The chemical nature of the sex pheromone was reported as aromatic compound with several atoms are protruded on the outer surface of the molecule. These atoms were suspected to have affinity towards the transmembrane protein of the male antennae and their sensory cells. (Fig.4).The possibility of the identified pheromone compound is 2 Butanone.

Fig. 1. Showing the Gas Chromatograph of the pheromones extracted from the cuticular abdominal glands of female American cockroach. Interestingly, the extracted substances contained nearly more than twenty compounds, of which around seven compounds found to be abundant in the extract. The fraction identified at 19th minute was observed to be 100% abundance. Numerous minor peaks were indentified in the extract which was suspected as the intermediate metabolites of the major fractionsof pheromone source.

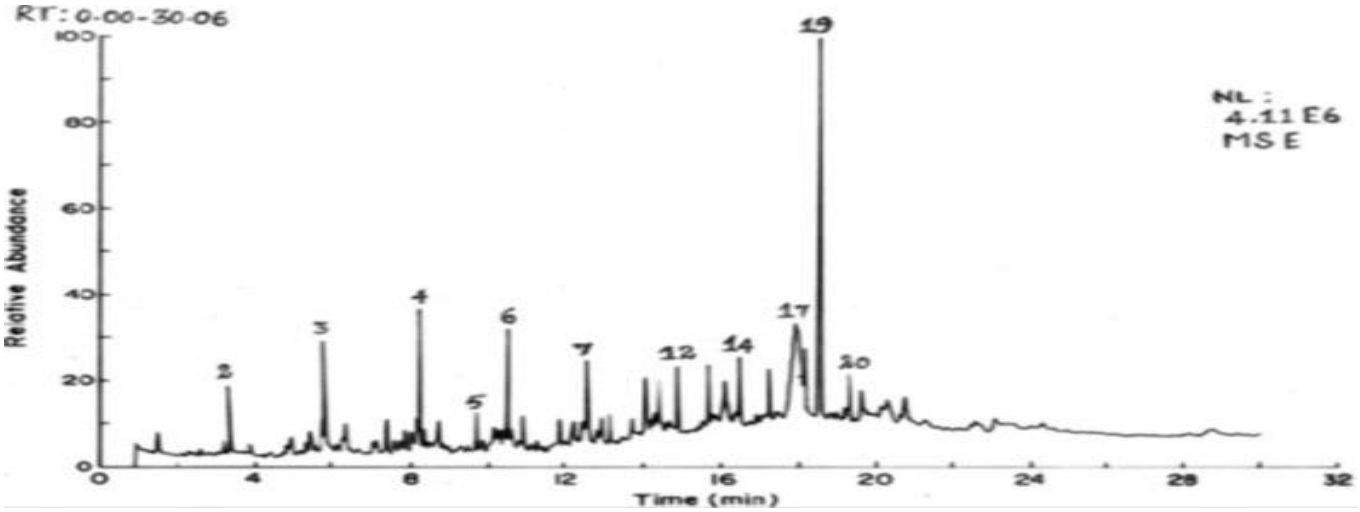


Fig. 2. Demonstrates the Infra Red Spectrum of the isolated pheromone compound which predominantly possessed the C=O (Keto) group with the peak value of 71715 and the hydrocarbon stretch was noticed with the peak value of 2991. Further, many more peaks were noted in the spectrum that suggested that the pheromone compound is not a single compound rather mixture of compounds or associated with their intermediate metabolites with the functional groups like aldehydes, alcohols and acids of both alkenes and alkanes.

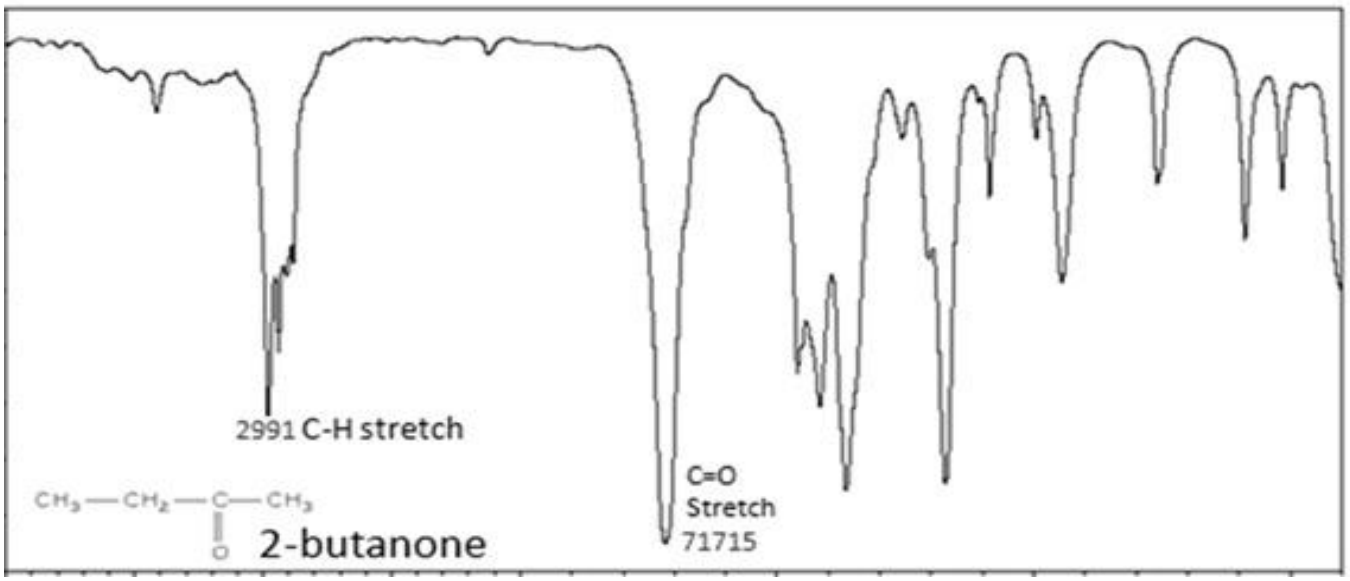


Fig. 3. Mass spectrum proves that the isolated and identified compound was 2-Butanone. The mass per charge values-76,132,151,164,178,181,207 and 237 were similar to the standard compound values available in the Chem-Library and its associated data base provided in instrumentation instruction manual.

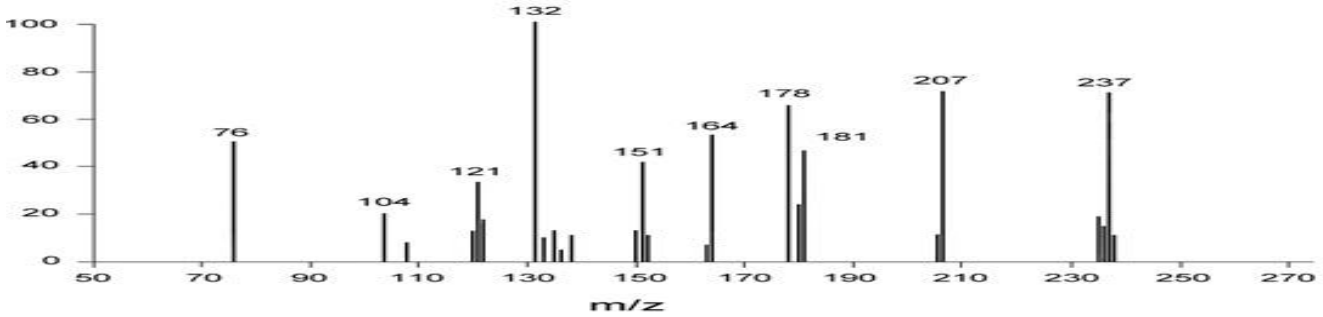
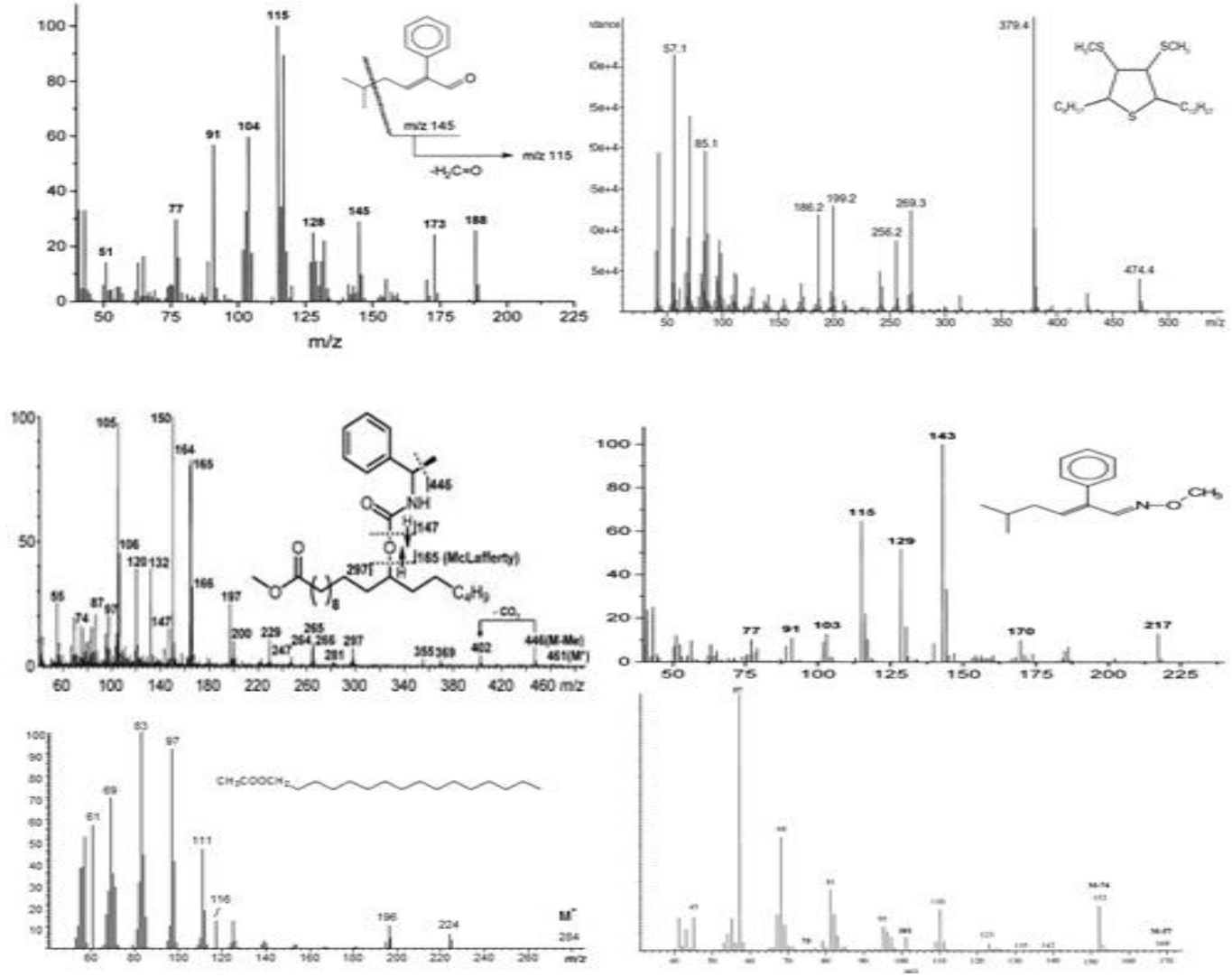


Fig. 4. The mass of each major peaks observed in the Gas Chromatograph were depicted here. This MS data clearly spell out that the sex pheromones of cuticular origin in American cockroach is a blend of both aliphatic and aromatic compounds. Mostly the chemical nature of the sex pheromone was reported as aromatic compound with several atoms are protruded on the outer surface of the molecule. These atoms were suspected to have affinity towards the transmembrane protein of the male antennae and their sensory cells. Thus, the Mass spectral analysis not only reveals the mass per charge ratio of the compounds but also provides a clue to hypothesise *i.e.*, the possibilities present in the pheromones to interact with its receptor in opposite sex.



DISCUSSION

The current investigation clearly proves that the female American cockroach contained many fractions in the cuticular regions which are all belonging to alkenes, alkanes of acids and ketones. In the case of cuticular abdominal gland of American cockroach, three major fractions which are chemically keto forms. Thus, the GC - MS and IR spectra experimentally authenticated that the sex pheromones of female American cockroach are hydrocarbon with ketone as functional group and some of the identified pheromones are fatty acids and aromatic acids. Similarly, the sex pheromone had been identified in the German cockroach. In the female German cockroach, *Blattella germanica* (L.) (Blattodea: Blattellidae), the volatile sex pheromone is produced in a gland located on the anterior of the last (10th) abdominal tergite gland [4].

The IR spectra of cuticular and scent gland reveals the occurrence of sex pheromones with keto groups. The female cupreous chafer, *Anomala cuprea* (Scarabaeidae), produces two lactone sex pheromone components: (*R,Z*)-5-(−)-(1-octenyl)oxacyclopentan-2-one and (*R,Z*)-5-(−)-(1-decenyl)oxacyclopentan-2-one. The biosynthetic route to these lactones involves the Δ9 desaturation of 16 and 18 carbon fatty acids, hydroxylation at carbon 8, two cycles of β-oxidation and cyclization [5]. The only step that is stereo specific is the hydroxylation step. Furthermore, the sex pheromones produced by female Lepidoptera are generally acyclic, fatty acid-derived compounds, 12 to 18 carbons in chain length with an oxygenated functional group (alcohol, aldehyde, or acetate ester) and zero to three double bonds [6]. In some cases, straight-chain or methyl-branched hydrocarbons have been shown to function as Lepidopteron pheromones. Variation in the chain length; the type of oxygenated functional group; the number, location, and isomeric nature of the double bond(s); and the precise ratios of components in multi-component pheromones collectively allow distinct, species-specific pheromone blends [7,8]. The cockroach pheromone components with double bonds have been noticed in the cuticular abdominal glands and cuticular substances using GC-MS analysis which gains support from the reports related to sex pheromones of lepidopteron species.

This MS data clearly spell out that the sex pheromones of cuticular origin in American cockroach is a blend of both aliphatic and aromatic compounds. Mostly the chemical nature of the sex pheromone was reported as aromatic compound with several atoms are protruded on the outer surface of the molecule. These atoms were suspected to have affinity towards the transmembrane protein of the male antennae and their sensory cells. Thus, the Mass spectral analysis not only reveals the mass per charge ratio of the compounds but also provides the possibilities present in the interaction of pheromones with its receptor in opposite sex.

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