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BEHAVIORAL RESPONSE OF RAT TO N-HEXYL-METHYL KETONE AND DECANOIC ACID DERIVATIVES IDENTIFIED FROM ARMPIT GLAND SECRETION

[1]Soundarapandian Kannan[*], [2]Govindaraju Archunan and [2]Ponnirul Ponmanickam

[1]Post Graduate and Research Department of Zoology, VHNSN College, Virudhunagar-626 001, Tamil Nadu, India
[2]Department of Animal Science, Bharathidasan University, Tiruchirappalli, 620 024, Tamil Nadu, India

Abstract: Armpit gland secretion from sexually mature and reproductively active male and female *Rattus rattus* (house rat), were analyzed by gas chromatography coupled mass spectrometry (GC-MS). Alkanes, aliphatic acids, esters and ketones were identified in the secretions. Four peaks were found to be higher concentration in male armpit gland which were identified as pentadecanoic acid 4,6,10,14 tetra methyl ester (I), decanoic acid (II), oleic acid (III) and decane 1,10 dibromo (IV) and where as the armpit gland of females three compounds viz., oleic acid (I), decane 1,10 dibromo (II) and n-hexyl-methyl ketone (III) were the major fractions. Odor preference test demonstrated that all the major chemical moieties identified in male armpit gland were found to attract both sexes. By contrast the III compound of female attracted conspecifics of the opposite sex, where compound I and II of females attracted both male and female individuals. The level of attraction also varied from compound to compound. The results conclude that the armpit gland of house rat contains five major fractions with pheromonal function in maintaining social behavior and reproductive status.

Keywords: Armpit Gland, GC-MS, Rat, Pheromonal Communication

INTRODUCTION

Nocturnal habits and dark living environments have led to the evolution of olfaction as a major method of communication in rodents (Robertson *et al*. 1993). Olfactory communication is essential to find energy and primary metabolites, avoid toxic substances and withdraw hostile environments. As regards animals, it is also vital for inter-individual communication among their same species, favor reproduction and social life organization. Pheromones are chemical signals, released in the medium, which improve biological process efficiency of congeneric animals, indicating to conspecific information of the sex, social and hormonal status of releaser. Any body secretions are potential routes for pheromonal communication and many mammals have well developed specialized scent glands that they use to deposit scent marks around their environment. All mammals emit chemical cues in their environment via urine, saliva or diverse secretion fluids (Briand *et al*. 2004; Brennan & Keverne 2004).

[*] Corresponding author: skbiochem04@yahoo.co.in

Specialized scent glands (Adams 1980; Balakrishnan & Alexander 1985; Kannan & Archunan 1997, 1998, 1999, 2000a, b, 2001), faeces (Asha *et al*. 1985) and urine (Johnston 1990; Selvaraj & Archunan 2002a) are reported to be the chief sources of mammalian pheromones.

As many as 40 different scent glands are identified in mammals (Balakrishnan & Alexander 1985), in which the pheromonal communication is well documented in some of the scent glands like preputial glands of mice (Marchlewska-Koj *et al*. 1990) and rat (Kannan & Archunan 2000a), cheek (Kannan & Archunan 1999) and clitoral glands (Kannan & Archunan 2001) of rat, flank glands of musk screw (Balakrishnan & Alexander 1977) and harderian gland of golden hamster (Bodyak & Surov 1994). It is generally believed that armpit gland produces pheromonal signals, which are likely to involve in bio-communication. However, the biological significance of the armpit gland is very scanty. The armpit gland is located under the forearm, in between armpit and lateral side of the chest. This gland is embedded in the armpit musculature, having bulging and depression on the surface, and number of storage vesicle (Kannan & Archunan 2000b). The gland secretes its substances through a number of minute hairs as projections, which are convincingly demonstrated in the laboratory rats (Kannan 1998) and lesser bandicoot rats (Kannan & Archunan 2000b).

Identifying the volatile compound(s) and assessing the behavioral response would be appropriate to confirm the glandular nature. Such chemical characterization study has been carried out in a number of species like mouse (Novotny *et al*. 1985), rat (Selvaraj & Archunan 2002b), tiger (Bramachary *et al*. 1992), bovine (Kumar *et al*. 2000) and elephant (Rasmussen & Lee 1991) to prove their behavioral significance. In the present study was carried out to characterize the glandular extract and to analyze its bioactivity in relation to pheromonal communication.

MATERIALS AND METHODS

Sexually mature and reproductively active adult male and female house rats were collected from nearby area and housed individually in polypropylene cages (40 × 25 × 15) cm with 2.0 cm of husk as bedding material. The bedding material was changed twice a week. Rat feed (Sai Durga Feeds & Foods, Bangalore) and water was provided *ad libitum*. Animals were maintained on a 14L:10D photoperiodic schedule in a climate controlled environment with a temperature of 25º ± 3ºC. Lights were on from 0600 and 1800 hours.

Twenty-five females in estrus and 25 male rats were sacrificed by cervical dislocation under mild diethyl ether anesthesia. After autopsy, armpit glands were dissected out under a dissection microscope and placed in double distilled solvent mixture (n-hexane and dichloromethane 1:1 ratio v/v) and ground well separately for about 10 minutes with glass homogeniser under ice-cold condition. The supernatant was filtered through silica gel (50–60 µ mesh size) and collected in a glass vial and sealed with air tight screw type cap made up of

glass (Borosil). The sample vials were stored at –20ºC until they were used for analysis.

Gas chromatography coupled mass spectrometry (GC-MS) analysis was made in a Shimadzu QP5000 (Japan) instrument under computer control at 70 eV. Ammonia was used as reagent gas at 95eV (Kannan 1998) performed chemical ionization. The identified compounds were fractionated by the method of Pause *et al*. (1997). The extracted samples (20 ml) were distilled for 5–10 minutes at room temperature (30ºC) under a vacuum of 0.2 torr. The distillate was condensed by cooling with liquid nitrogen and concentrated to 2 ml. The volatiles form the distilled fractions were subjected to gas chromatography for cross checking and confirmation of compounds in each fraction (Pause *et al*. 1997).

Assuming the importance of compounds in pheromone activity, the odor preference test was conducted in the Y-maze apparatus with the modified procedure of Ferkin and Seamon (1987). The Y-maze apparatus was made up of tin sheet, which consists of a central arm and two choice arms. The lateral sides were closed with glass plates where as top portion was covered with wire meshes. This apparatus had facility to provide food and water *ad libitum*. The size of central arm was about 80 cm length and 15 cm width. The remaining two choice arms were 75 cm length and 15 cm width each. The glandular extract slide was placed on the extreme right arm and the solvent slide (control) was placed on the extreme left arm during behavioral analysis. The position of the odor (left or right choice arms) was alternated. Three individuals (either male or female) were randomly taken from a pool of rats kept separately for each sex and used for each behavioral analysis. Test animals were released in the central arm and their behavior was assessed for 15 minutes with the identified compounds (experimental) and the solvent mixture was used as control. Each animal was tested thrice against individual compound, making a total of nine observations on each set. The time taken for visiting each compound was recorded. The data were analyzed by student's t-test.

RESULTS

As shown by the results summarized in Table 1 and 2, the following compounds were found in male armpit gland secretions. They were pentadecanoic acid 4,6,10,14, tetra methyl ester (I), decanoic acid (II), oleic acid (III) and decane 1, 10 dibromo (IV). It was very interesting to note that all the four volatile fractions of male armpit gland significantly attract both male and female rats (Table 2). Similar chemical identification study was performed using female armpit gland, which showed that it had three major volatile compounds namely oleic acid (I), decane 1,10 dibromo (II) and n-hexyl-methyl ketone (III).

Four identified compounds of male armpit gland namely, pentadecanoic acid 4,6,10,14 tetra methyl ester (I), decanoic acid (II), oleic acid (III) and decane 1,10 dibromo (IV) effectively attracted the individuals of both sexes belonging to the same species. Similar study was carried out in female which revealed that the male and female responders spent greater time towards all the identified

compounds except compound III of female armpit gland. Rather the third compound, n-hexyl-methyl-ketone attracted the opposite sex (Table 1 and 2).

Table 1: List of chemical compounds identified from armpit glands of male and female *Rattus rattus* (house rats).

Serial number	Name of the identified compounds	Mass	Male	Female	Biological significance
1	Decanoic acid	172.26	II	-	Both sex attractant
2	Decane 1,10 dibromo	300.07	IV	II	Both sex attractant
3	Oleic acid	282.46	III	I	Both sex attractant
4	Pentadecanoic acid 4,6,10,14 tetra methyl ester	420.80	I	-	Both sex attractant
5	n-hexyl-methyl ketone	142.23	-	III	Male attractant

Table 2: Responses of male and female house rats to various identified compounds of armpit gland (in seconds).

	I	Blank	II	Blank	III	Blank	IV	Blank
Mag SS	267.14 ± .31#	29.71 ± 1.88	307.28 ± 4.98#	28.57 ± 2.53	208.57 ± 6.00#	29.50 ± 2.32	231.50 ± 4.51#	27.12 ± 1.50
Mag OS	262.57 ± 5.69#	27.83 ± 1.92	298.42 ± 4.68#	26.00 ± 1.63	193.33 ± 3.58	28.83 ± 1.99	403.85 ± 6.89**	27.16 ± 2.76
Fag SS	291.50 ± 5.44#	28.50 ± 1.76	182.50 ± 3.02#	37.70 ± 2.01	256.71 ± 3.78*	27.85 ± 1.88	-	-
Fag OS	301.50 ± 6.12#	33.14 ± 1.69	202.57 ± 6.67#	26.14 ± 2.70	398.70 ± 4.46#*	28.14 ± 2.22	-	-

± Standard error of mean for six observations
* 95% significant at P = 0.05 as compared between SS and OS (opposite sex attractant)
# 95% significant at P = 0.05 as compared between blank slide (both sex attractant)
Mag – Male armpit gland
Fag – Female armpit gland
SS – Same sex
OS – Opposite sex

DISCUSSIONS

In the present investigation, five major volatile compounds are identified as major constituents in both male and female armpit glands extract (Fig. 1 & 2). In female rats, the chemical identification was performed during estrus period only because it is found to be the period of releasing chemical signals and their perception. Similar observation was made in white and tailed deer's vaginal fluid and urine (Jemiolo *et al*. 1995) and in house mouse's urine (Andreolini *et al*. 1987). Further, the estrus female discharged a mucous that was richer in the characteristic volatiles than the females in mid-cycle. Estrus female, but not mid-cycle secretions of female, has been shown to elicit courtship behavior among male white tailed deer (Dominic 1991; Johnston 1990; Whitney *et al*. 1992; Murphy *et al*. 1994). The above results are consistent with our findings.

Behavioral observation clearly reveals that the compound present in the male armpit gland is found to attract both sexes. Therefore, it indicates that four compounds produced from armpit gland of male maintain normal social activity of individual. By contrast, three compounds are characterized in female armpit glands and 1 compound, n-hexyl-methyl ketone act as a male sex attractant. It is evident that the odor required to attract the male from armpit sources is complex in nature. The secretory compounds are synthesized in the secretory cells and liberated through the osmatrichia (which transfer these compounds from cells) to the environment for its significant function (Kannan & Archunan 2000b). Through the present behavioral assessment, armpit gland appears to be an important site for pheromonal production to attract conspecifics. Experimental evidence in human (Zeng *et al*. 1996) also shows the human axillaries extracts alter the length and timing of menstrual cycle.

Several reports are available regarding the significance of pheromone trap in insect pest management (Cork *et al*. 2003; Cork & Hall 1998). The insect pheromone traps are commercially available to reduce the insect pest menace in agricultural crops. However, control of rodent population is not effective to our expectations, as there are always problems. Moreover, the study on the usage of mammalian pheromone in rodent pest management is very limited. Nevertheless, the recent study in our laboratory showed that scent gland extracts are capable of improving poison bait acceptance and increase the rate of mortality in *Rattus norvegicus* (albino rat) (Selvaraj & Archunan 2002b). Developing pheromonal trap would be the best method of rodent pest management program. Hence, identifying the rodent pheromones would definitely provide a strong foundation to introduce a novel and more achievable technique for rodent pest management programs.

From this investigation, it could be concluded that the possibility of specific olfactory signals can be produced by more than one gland. Further, it suggests that the odor produced by more than one gland act together to manifest the specific pheromonal function. The present findings clearly emphasize that in addition to other scent glands, the armpit gland also faithfully involved in conveying social signals between the individuals.

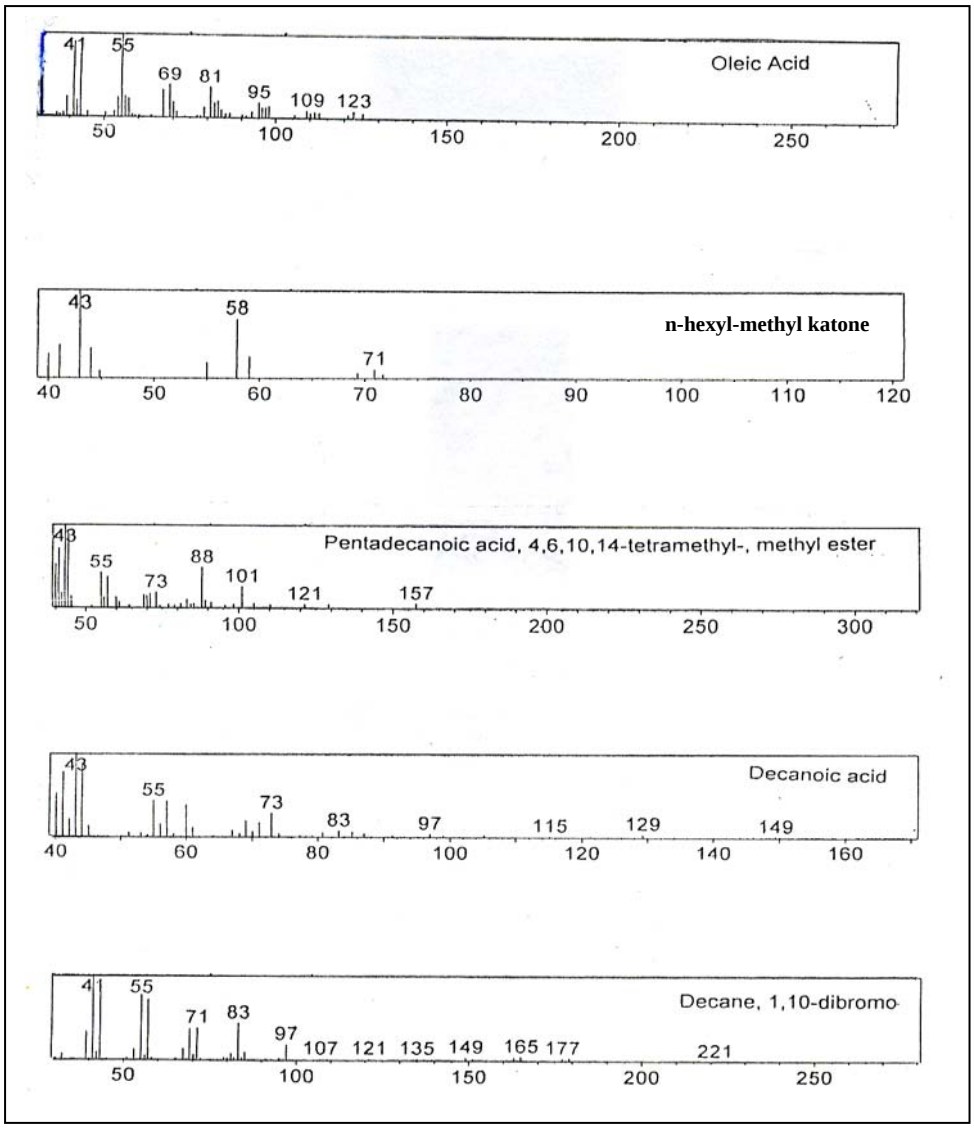

Figure 1: Mass spectra for the identified compounds of male and female armpit glands of *Rattus rattus* (house rat).

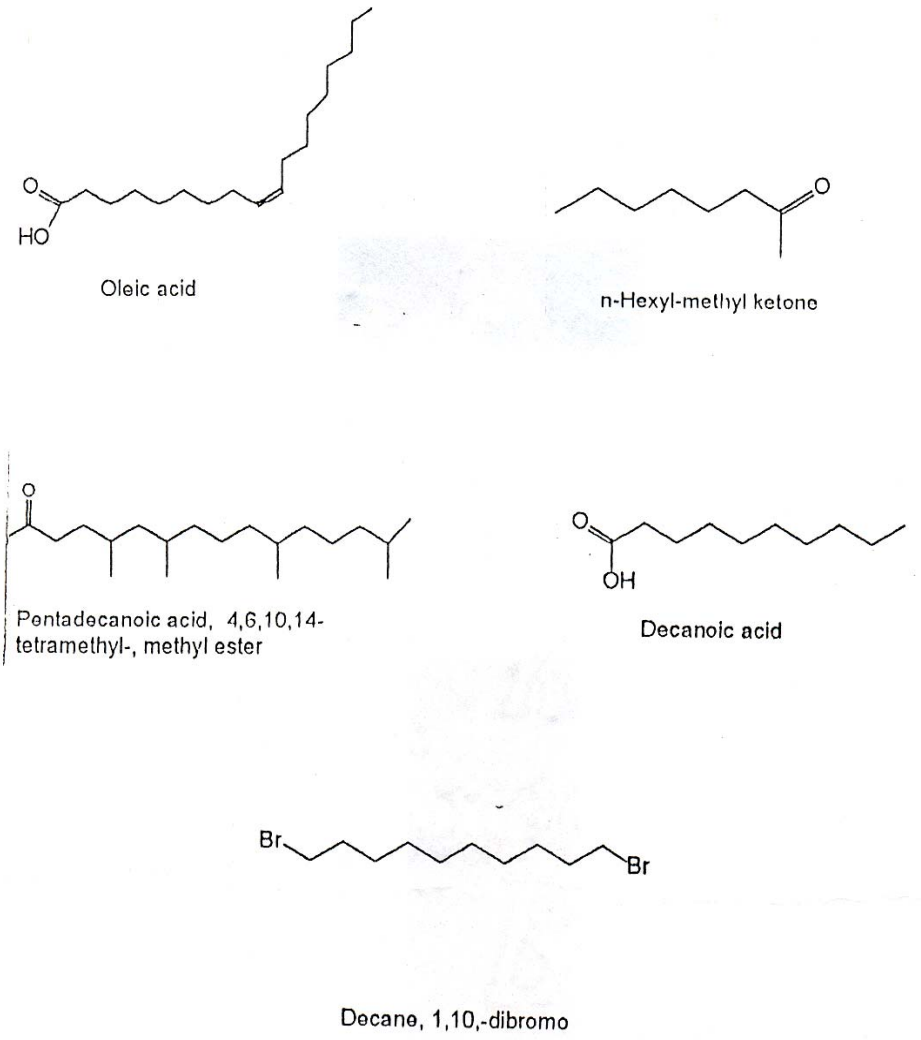

Figure 2: Chemical structure of the compounds identified from armpit glands of male and female *Rattus rattus* (house rat).

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