full moon nights under canopy and that it hardly foraged.

1. Balasingh, J., John Koilraj, A. and Kunz, T. H., *Ethology*, 1995, **100**, 210–229.
2. Balasingh, J., Isaac S. S. and Subbaraj, R., *Curr. Sci.*, 1993, **65**, 418.
3. Marimuthu, G., Rajan, K. E., John Koilraj, A., Suthakar Isaac, S. and Balasingh, J., *Biotropica*, 1998, in press.
4. Kunz, T. H., in *Ecological and Behavioral Methods for the Study of Bats* (ed. Kunz, T. H.), Smithsonian Institution Press, Washington D.C., 1988.
5. Bower, M. A., *Ecology*, 1990, **71**, 2334–2344.
6. Kotler, B. P., Brown, J. S., Smith, R. J. and Wirtz, W.O., *Oikos*, 1988, **53**, 145–152.
7. Longland, W. S. and Price, M. V., *Ecology*, 1991, **72**, 2261–2273.
8. Julien-Laferriere, D., *J. Mammal.*, 1997, **78**, 251–255.
9. Morrison, D. W., *Anim. Behav.*, 1978, **26**, 852–855.
10. Vaughan, T. A., *Afr. Wildl. J.*, 1977, **15**, 237–249.
11. Usman, K., Habersetzer, J., Subbaraj, R., Gopalakrishnaswamy, G. and Paramanandam, K., *Behav. Ecol. Sociobiol.*, 1980, **7**, 79–81.

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Sex attractants in male preputial gland: Chemical identification and their role in reproductive behaviour of rats

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We report here the structural elucidation of three hitherto unreported pheromonal components unique to male rats. The structures are (i) 2,6,10-dodecatrien-1-ol,3,7,11-trimethyl (Z, E); (ii) Di-*n*-octyl phthalate, and (iii) 1,2 Benzenedicarboxylic acid, diisooctyl ester. Of these, the first two attract the opposite sex while the third compound attracts the same sex.

CHEMICAL signals appear to play an important function in the overall social behaviour and reproductive behaviours of rodents[1–3]. The rat, *Rattus norvegicus*[3] has a variety of known and potential sources of sex attractants such as various integumentary glands including preputial glands and excretory substances namely urine and faeces[2]. However, the excretory material gets the pheromonal compounds from the secretions of subcutaneous glands. The glandular secretions might be bye-passed into the urinary tract and/or external genitalia. For instance, the preputial glands situated between the dermis and body wall anterior to the penis in males and to clitoris in females seem to be a source of pheromones and release part of their secretions through urine[4].

These glands are prominent in males but are comparatively smaller in females[5]. The degree of male preputial gland activity is apparently related to social experience, particularly aggression[6]. Preputial gland secretions are known to attract the opposite sex in white-tailed deer[7]. They may also regulate anogenital licking and aid in maternal discrimination of the sex of pups[8]. de Catanzaro *et al.*[9] reported that male preputial and salivary glands are sources of pheromones that stimulate oestrus in female mice, but preputial gland secretions are not involved in the male-induced pregnancy block[9].

Chemical identity of mammalian pheromones such as the urinary compounds of mice[10], tiger[11], and elephant[12] is available. The significance of male preputial glands in social and sexual behaviour of rats has been investigated and the involvement of the preputial odour in eliciting females' or intermales' aggression has been reported[7]. However, chemical identification of pheromonal compounds of preputial gland secretion in male rats and their functions in social and sexual behaviour are still obscure. The present report deals with the chemical nature of male preputial glands in rats.

Sexually matured and reproductively active laboratory rats, *Rattus norvegicus* weighing 200–250 g were maintained on a 12:12 light:dark cycle in the laboratory. The animals were given rat feed and tap water *ad libitum*. The animals were killed by cervical dislocation. As sufficient quantity of the preputial secretion of rats was difficult to collect, the preputial glands were removed from 20 rats and ground well in a mixture of solvents (methylene chloride and *n*-hexane 1:1 v/v) at room temperature. The supernatent was then filtered through silica gel column (60–120 mesh) and concentrated under vacuum (temperature <30°C) for fractionation and chemical identification using gas chromatography-linked mass spectrometric (GC–MS) analyses by comparison with standard compounds. The GC–MS analyses were made in a Nermag R-10-10C instrument under computer control at 70 eV. Chemical ionization was performed by using ammonia as reagent gas at 95 eV (ref. 13). Then the chemical mixtures were subjected for fractionation to separate the compounds.

Assuming the importance of the fractionated compounds in pheromonal activity, Y-Maze odour preference test was conducted by following the procedure of Ferkin and Seamon[14]. In three different sets of same and

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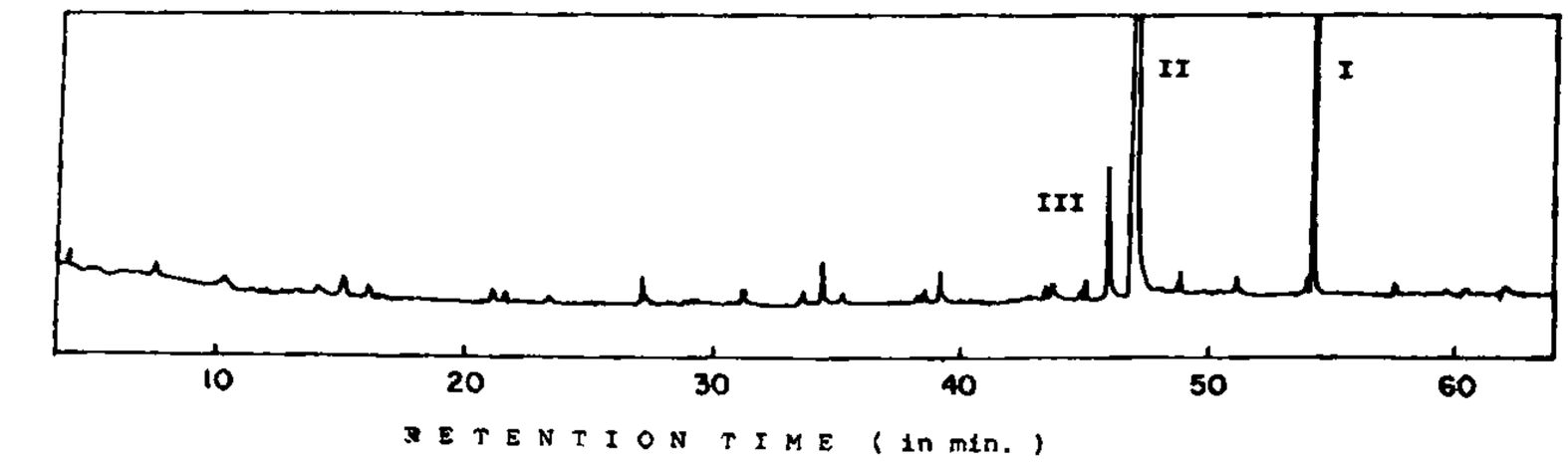

Figure 1. Gas chromatographic profiles of sex attractants from preputial glands of male albino rats *Rattus norvegicus*. I, 2,6,10-Dodecatrien-1-ol,3,7,11-trimethyl (Z, E); II, Di-*n*-octyl phthalate; III, 1,2,benzenedicarboxylic acid isooctyl ester.

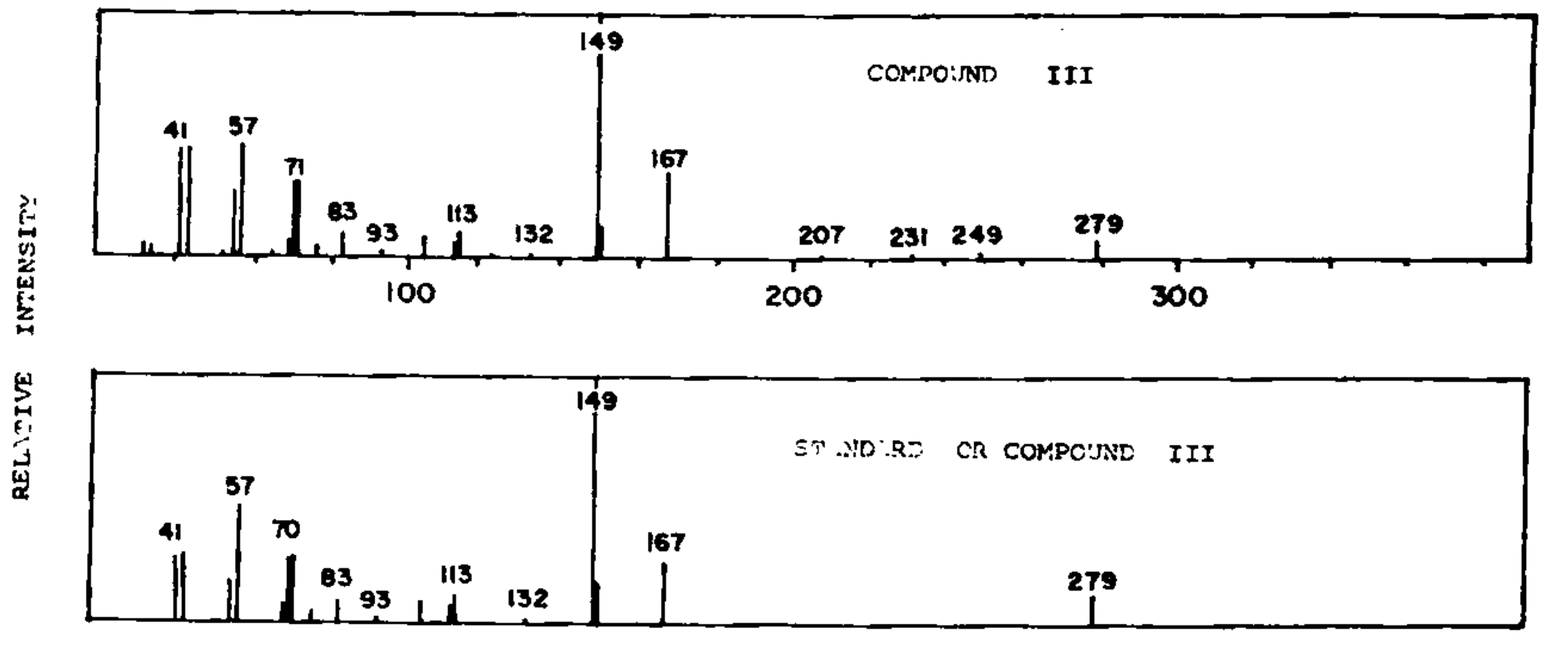

Figure 2. Relative concentrations of the III compound (1,2,benzenedicarboxylic acid isooctyl ester) identified in dichloromethane + *n*-hexane extract of preputial gland secretions from male rats (mass spectrum).

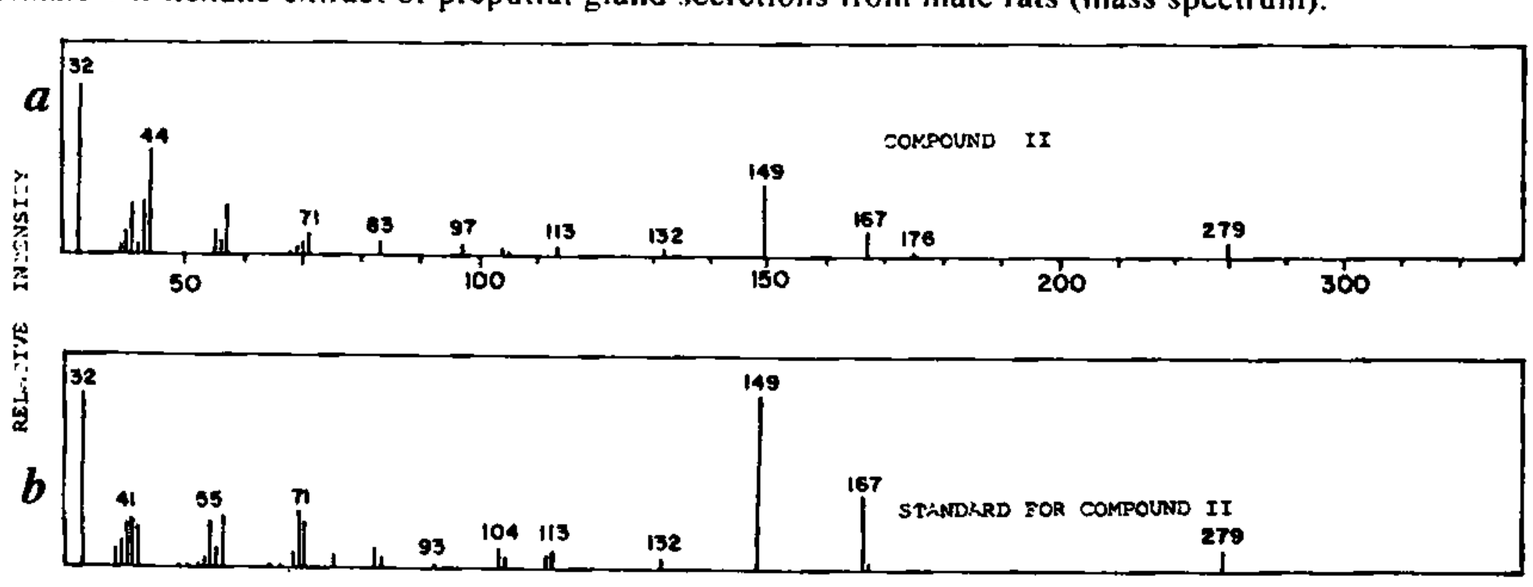

Figure 3. Electron impact-mass spectra of (*a*) compound II (di-*n*-octyl phthalate), (*b*) Standard for compound II isolated from male preputial gland.

Table 1. Bioassay responses of male and female rats to the three different fractions of male preputial gland secretions

Responder	Time (in seconds) spent by the individual towards the fractions of male preputial gland extract for 15 minutes test (trails: 3 times/set)					
	I	Blank slide	II	Blank slide	III	Blank slide
Same sex (n = 3/set)	107.6 ± 3.25	31.0 ± 1.98	115.6 ± 3.73	33.83 ± 1.73	386.2 ± 6.49*	36.8 ± 1.56
Opposite sex (n = 3/set)	412.6 ± 4.68*	37.0 ± 2.31	389.8 ± 5.02*	34.0 ± 1.31	117.5 ± 3.09	28.33 ± 4.49

±represents the standard error of mean.

*Level of significance at $P < 0.005$ compared with blank (Student's t test).

I, 2,6,10-Dodecatrien-1-ol,3,7,11-trimethyl (Z, E); II, Di-*n*-octyl phthalate; III, 1,2 Benzenedicarboxylic acid, diisooctyl ester.

Level of significance in same sex.

I vs II NS; I vs III significant at $P < 0.005$ level; II vs III significant at $P < 0.005$ level.

Level of significance in opposite sex.

I vs II significant at $P < 0.05$ level; I vs III significant at $P < 0.005$ level; II vs III significant at $P < 0.005$ level.

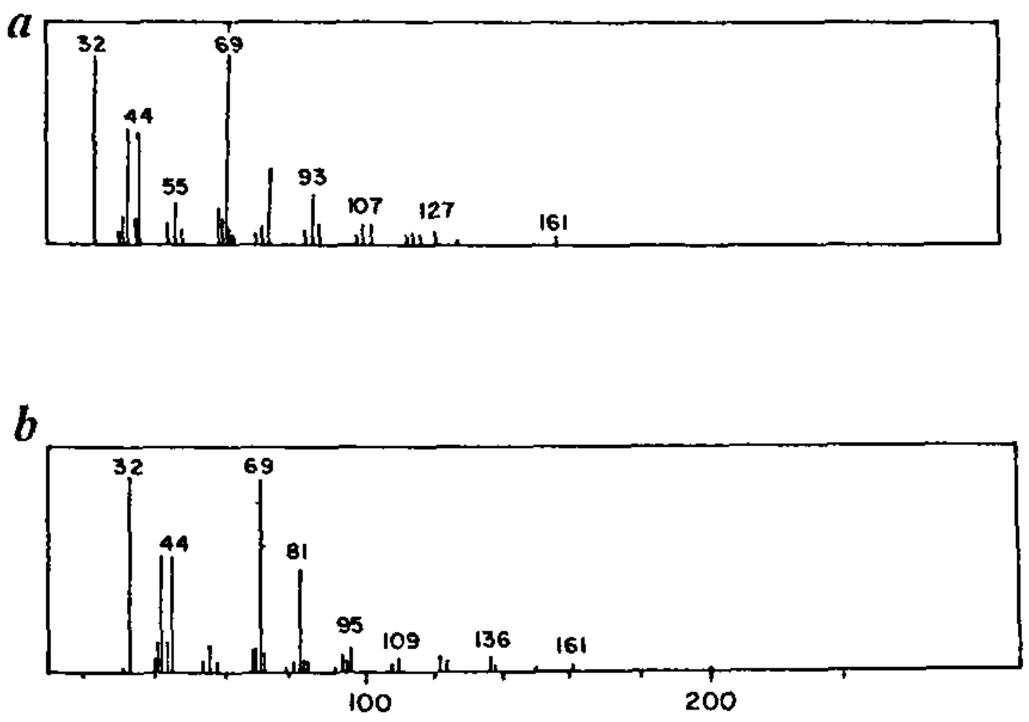

Figure 4. Electron impact-mass spectra of (*a*) Compound I (2,6,10-dodecatrien-1-ol,3,7,11-trimethyl (Z, E) and (*b*) standard for compound I isolated from male preputial gland.

opposite sexes, 3 animals were used in each set for behaviour analysis. Fresh samples were used in each trial. The behaviour was assessed for 15 min with these compounds and the solvents alone as control. The responders were of the same and the opposite sex. The time taken for visiting each fraction was recorded and subjected to statistical analyses.

The extracted male preputial gland contains 2,6,10-dodecatrien-1-ol,3,7,11-trimethyl (Z, E), di-*n*-octyl phthalate and 1,2,benzenedicarboxylic acid, diisooctyl ester (Figures 1–4). These chemical substances have their unique functions. The first two compounds are involved in the attraction of the opposite sex and the third compound is involved in the attraction of the same sex (Table 1). Thus, the preputial gland of male rat contains three different compounds with distinct social functions.

1. Dominic, C. J., *Ad. Bios.*, 1986, **4**, 71–86.
2. Balakrishnan, M. and Alexander, K. M., *Indian Rev. Life Sci.*, 1985, **5**, 277–313.
3. Kannan, S. and Archunan, G., *Rev. Clenc. Biomed.*, 1997, in press.
4. Asa, C. S., Mech, L. D. and Seal, U. S., *Anim. Behav.*, 1984, **33**, 1034–1036.
5. Kannan, S. and Archunan, G., *Acta Physiologica*, 1997, in press.
6. Thody, A. J. and Dijkstra, H., *J. Endocrinol.*, 1978, **77**, 397–403.
7. Karl, S. O., Miller, V. and Hoffmann, D. M., *J. Mamm.*, 1992, **73**, 299–302.
8. Brouette-Lahlou, I., Amouroux, R., Chastrette, F., Cosnier, J., Stoffelsma, J. and Vernet-Maury, E., *J .Chem. Ecol.*, 1991, **17**, 1343–1354.
9. de Catanzaro, D., Zacharias, R. and Muir, C., *J. Reprod. Fertil.*, 1996, **106**, 269–274.
10. Andreolini, F., Jemiolo, B. and Novotny, M., *Experientia*, 1987, **43**, 998–1002.
11. Brahmachary, R. L., Podder-Sarkar, M. and Dutta, J., *Nature*, 1990, **344**, 26.
12. Rasmussen, L. E. L., Lee, T. D., Roelofs, W. L., Zhang, A. and Daves, G. D., *Nature*, 1996, **379**, 684.
13. Tannoudji, J. C., Einhorn, J. and Signoret, J. P., *Physiol. Behav.*, 1994, **56**, 955–961.
14. Ferkin, M. H. and Seamon, J. O., *Can. J. Zool.*, 1987, **65**, 2931–2937.

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Effect of chronic atenolol treatment on responses to noradrenaline and terbutaline in isolated ventricle from hypertensive and hyperthyroid rats

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The present investigation was undertaken to study the effect of chronic treatment with atenolol on noradrenaline and terbutaline in hypertensive and hyperthyroid rats. The maximum increase in force of contraction of ventricle by noradrenaline in control preparations chronically treated with atenolol was reduced, while that produced by terbutaline was not affected. The pD_2 values of both the agonists were increased. In DOCA-saline hypertensive preparations, chronic treatment with atenelol did not produce any change in noradrenaline-induced positive inotropic responses; however, the maximal response to terbutaline was reduced. While the pD_2 value of noradrenaline was decreased, that of terbutaline was increased. Chronic treatment with atenolol in hyperthyroid rats did not produce any significant effect on the maximal contractions of ventricle with either noradrenaline or terbutaline. However, the pD_2 value of noradrenaline was reduced while that of terbutaline was unaffected. It is concluded that the beneficial effects of atenolol in hypertension and hyperthyroidism may be related to reduction in the number of beta-receptors in the heart.

BETA$_1$- and beta$_2$-adrenoceptors co-exist in the hearts of most species, with the beta$_1$-adrenoceptor predominating functionally under normal physiological conditions[1–3]. Chronic administration of agonist or antagonist receptor ligands results in decrease (down-regulation) and increase (up-regulation) respectively in ligand-binding

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