

# Amines in the Marking Fluid and Anal Sac Secretion of the Tiger, *Panthera tigris*

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Analysis of the marking fluid of two tigers (one Bengal and one Sumatran) by GC using an amine-specific column and a nitrogen-specific detector has shown the presence of the following amines: ammonia, methylamine, dimethylamine, trimethylamine, triethylamine, propylamine, and butane-1,4-diamine (putrescine). In contrast to previously published reports, we were unable to detect 2-phenylethylamine. The anal sac secretion was found to have a similar amine content.

## Introduction

Tigers use a variety of scent-markings in the wild. Smith *et al.* [1], on the basis of their field studies of tigers in Nepal, list several modes of scent-marking: scrapes, scats, anal sac secretion, urine stains on trees and shrubs, claw-raking and cheek-rubbing. The major purpose of these markings seems to be for the establishment and maintenance of territory [1]. Another form of scent-marking, not mentioned explicitly in the above report, has been described by Schaller [2] and by Brahmachary and Dutta [3–5], and is considered by the latter authors to be semiochemically the most significant method of marking. This comprises a white fatty fluid, termed ‘marking fluid’ [3–5], which is sprayed at an angle as a jet, independent of urine, from the urinary tract of both male and female tigers when the hind leg is raised [4]. This action deposits the marking fluid on vegetation 1–1.3 m above the ground.

As pointed out [5], there has been some confusion in the literature between anal sac secretion and marking fluid, even though they are distinctly different in appearance and origin. Deposits of anal sac secretion are described as being dark and waxy with a distinctive odour [1], whereas marking fluid is white and much more mobile with an ammoniacal odour. Anal sac secretion is deposited with faeces; and as there is no connection between the anal gland and the urinary tract, there is no

way in which anal gland secretion can be emitted with urine. Urine, on the other hand, may contain small amounts of marking fluid [4].

Analysis of steam distillate extracts of marking fluid from both male and female tigers using TLC and paper chromatography has been carried out [3, 4]. The chromatograms revealed the presence of several amines, of which 2-phenylethylamine was reported to be the major component in each animal. Other amines identified were butane-1,4-diamine (putrescine) and pentane-1,5-diamine (cadaverine) as well as ammonia [5]. No analytical work on the anal sac secretion of the tiger has yet been published.

As part of our long-term interest in the composition of semiochemically important glandular secretion of mammals, we have analyzed both the marking fluid and anal sac secretion of the tiger specifically for the presence of amines, and we present here our preliminary findings.

## Materials and Methods

Marking fluid from two female tigers, Kiawang (Bengal) and Suzy (Sumatran), were collected from their enclosures in London Zoo, Regent's Park. Anal sac secretion from tiger Kiawang was obtained directly from the anal sac while the animal was anaesthetized. All samples were stored at –15 °C until analyzed. Analyses were carried out on a Varian 3700 gas chromatograph equipped with a glass column (2 m long, 2 mm ID) packed with 60/80 Carbowax B/4% Carbowax 20 M/0.8% KOH (Supelco). The detector was a thermionic detector for the specific detection of nitrogen. Samples (~100 mg) of marking fluid and anal

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sac secretion were dissolved in 0.05 M NaOH solution (1.0 ml), and 4  $\mu$ l injected onto the column. The column temperature was held at 50 °C for 2 min, and was then raised to 210 °C at 50 °C/min. The nitrogen flow rate was 30 ml/min.

## Results and Discussion

The gas chromatogram from a sample of marking fluid from Kiawang (collected 7.11.89) is shown in Fig. 1. The identities of the amines were determined by comparison of retention times with those of authentic samples. The major amine in the secretion is trimethylamine, with a significant amount of butane-1,4-diamine and smaller amounts of methylamine, dimethylamine and triethylamine. In stark contrast to previously published work [3, 4], the chromatogram shows only a possible hint of 2-phenylethylamine at a retention time of 12 min. To check that we had not missed this amine, 2-phenylethylamine (0.5 mg) was added to a sample of marking fluid (100 mg) and the mixture analyzed under the same conditions. The resulting chromatogram is shown in Fig. 2, in which the large peak due to 2-phenylethylamine has a retention time of 12 min. This result clearly shows that 2-phenylethylamine is not present in this sample of secretion to any significant extent. A second sample of secretion taken from the same tiger at a later date (21.2.91) revealed the same suite of amines, the main difference between the

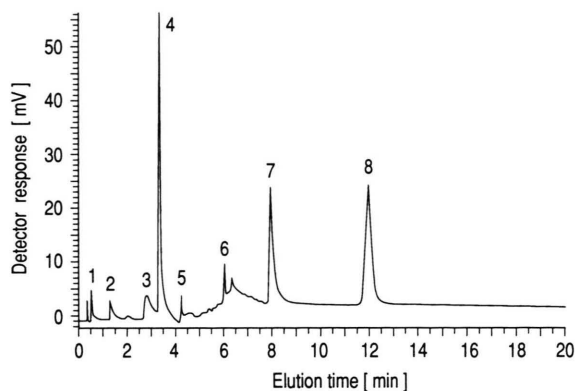


Fig. 2. Gas chromatogram of marking fluid from tiger Kiawang collected 7.11.89 with added 2-phenylethylamine (compound 8). Compounds 1–7 as in Fig. 1.

two samples being the reduced amount of butane-1,4-diamine in the latter (Fig. 3). The reason for this difference is not certain, but it does indicate that the composition of secretion from an individual does vary. The chromatogram obtained from the marking fluid of tiger Suzy is shown in Fig. 4. This is almost identical to the chromatogram of the first sample taken from Kiawang, despite the fact that the animals were from different subspecies, and again, there is no indication of 2-phenylethylamine.

Fig. 5 shows the chromatogram obtained from the anal sac secretion of Kiawang. Although this

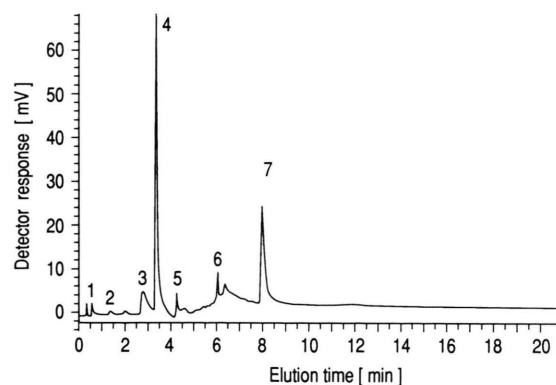


Fig. 1. Gas chromatogram of marking fluid from tiger Kiawang collected 7.11.89. Compounds identified as follows: 1, ammonia; 2, methylamine; 3, dimethylamine; 4, trimethylamine; 5, propylamine; 6, triethylamine; 7, butane-1,4-diamine.

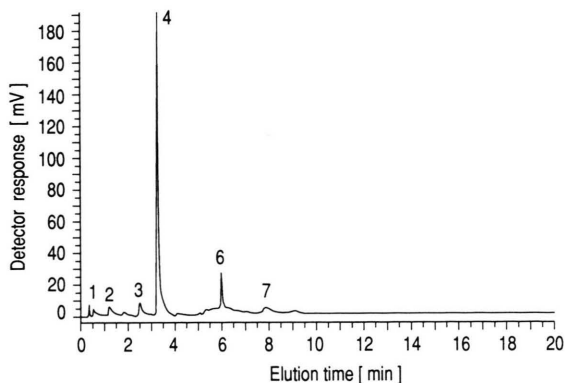


Fig. 3. Gas chromatograph of marking fluid from tiger Kiawang collected 21.2.91. Compounds 1–7 as in Fig. 1.

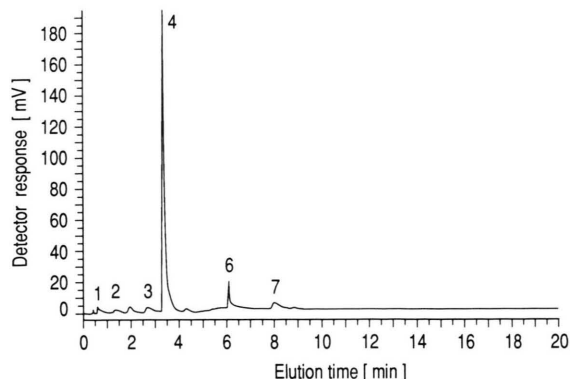


Fig. 4. Gas chromatogram of marking fluid from tiger Suzy. Compounds **1**–**7** as in Fig. 1.

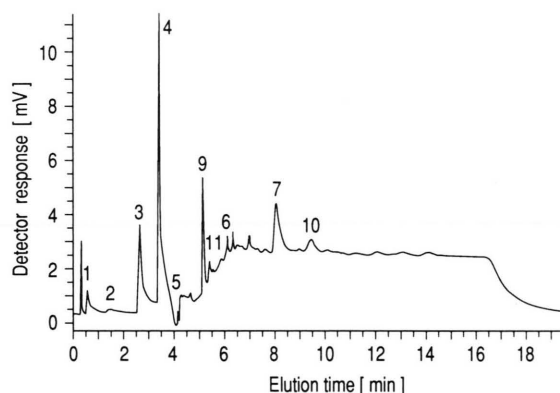


Fig. 5. Gas chromatograph of the anal sac secretion of tiger Kiawang. Compounds **1**–**7** as in Fig. 1; **9**, iso-butylamine; **10**, pentane-1,5-diamine; **11**, butylamine.

dark, waxy material is so different in its appearance from the white, mobile marking fluid, it has an amine profile that is strikingly similar to that of the latter. One of the few differences is the presence of a significant amount of iso-butylamine in the anal sac secretion. Again, 2-phenylethylamine was not observed.

The great similarity in the amine contents of the marking fluid and anal sac secretion was unexpected and surprising, and it raises the question of the relationship between these two excretions, seemingly so different in both appearance and mode of deposition. Do they carry the same semiochemical message? It is also difficult to understand the great discrepancy between our results and previously published reports [3–5] concerning the presence of 2-phenylethylamine in the marking fluid. Although our work and previous studies [4] show that some variation is to be expected between individuals and over time for the same individual, the dichotomy regarding 2-phenylethylamine would seem to be too great to be explained in these terms. It may be that the absence of 2-phenylethylamine in our tigers reflects a metabolic difference between them and the tigers previously studied in India [3–5].

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