Chemical Composition of the Secretion from the Anal Sacs of *Civettictis civetta* (Schreber, 1776)

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The chemical composition of the secretion from the anal sacs of a female of *Civettictis civetta* is analysed using gas-liquid chromatographical and mass spectrometrical techniques. The secretion mainly consists of cholesterol esters, monoester waxes, cholesterol, and free fatty acids of chain lengths not under C_{12}; highly volatile components were not traceable. The functional significance of the anal sac secretion is discussed.

Introduction

The functional importance of pheromones for communication and their role in the social life of mammals has been proved by many authors. Mainly through behavioural studies this has also been shown to be true for viverrids [1–8]. Many of these studies were focused on scent marking behaviour and consequently, the use of the perineal glands and the anal sacs, the most important glandular organs of viverrids for producing chemical signals, has been reported upon.

Only very few references on the morphology of the organs and the chemical composition of the secretions, however, are available for viverrids. Substantial information on the structure of the anal sacs and also partially on the perineal glands are given by Pocock [9, 10], Schaffer [11], and Ortmann [12]. Recently Gorman *et al.* [4] have been working on the morphology of the anal sacs of *Herpestes auropunctatus* while Kayanja and Schliemann [13] reported on the histology and ultrastructure of the sebaceous glands of the anal sacs of *Genetta trigrina*. In the secretion of the anal sacs of *Herpestes*, Gorman *et al.* detected some homologous and isomeric free short-chain fatty acids the formation of which is suppressed by penicillin.

Civettictis civetta, the African civet, is a large terrestrial and mainly nocturnal viverrid the wild biology of which is not yet well known. However, Ewer and Wemmer [3] were able to study the behaviour of captive specimens of *Civettictis*. Additional information on the ethology of the civet including scent marking behaviour is given by Ewer [14], Rosevear [15], and Wemmer [7]. According to these authors it is evident that mainly the perineal organ is used for scent marking, a fact confirmed in connection with the present work. Not yet clarified, however, is the functional significance of the anal sacs not only of *Civettictis* but also of all those viverrids which possess both organs, *i.e.* perineal glands and anal sacs. Presumably further ethological, morphological, and chemical studies would contribute to answer the question concerning the anal sac function. The present paper giving the chemical composition of the anal sac secretion of *Civettictis civetta* is thought to improve the understanding of the skin glands of viverrids.

Materials and Methods

Materials

Secretion was obtained from an adult female of *Civettictis civetta* (Schreber, 1776) under light anaesthesia by squeezing the anal sacs. The animal which had been collected in Liberia and which had been kept for several years in the Bernhard-Nocht-Institut für Schiffs- und Tropenkrankheiten at Hamburg is now living in the animal house of the Zoologisches Institut und Zoologisches Museum/ Hamburg.

Methods

After distribution between chloroform/methanol/water (2:1:1; v/v; 60 ml) the crude lipids (128 mg) remain in the chloroform phase. According to comparison with authentic reference substances they proved to be a complex mixture of cholesterol esters, mono- and diester waxes, triglycerides, free alcohols, cholesterol, and free fatty acids. The free

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fatty acids (32.9 mg) were separated with methanolic NaOH as Na-salts and recovered by acidifying using HCl and extraction with $CHCl_3$. The remaining lipid mixture was separated by SiO_2-column chromatography (5 g silica gel, 14.5% water content) using a $C_6H_{12}/C_6H_6/CHCl_3$ gradient [16]. Cholesterol esters (20.6 mg) and monoester waxes (28.5 mg) were separated with cyclohexane/benzene (9:1; v/v; 100 ml), diester waxes (5.0 mg) with cyclohexane/benzene (9:6; v/v; 75 ml), triglycerides (7.3 mg) with benzene (70 ml), and free alcohols (3.7 mg) together with cholesterol (29.8 mg) with benzene/chloroform ((1:1; v/v; 50 ml). The free fatty acids were esterified and the cholesterol esters, the monoester waxes and triglycerides were re-esterified with 5% methanolic HCl (20 ml; 45 min) at boiling-point temperature. After adding water (20 ml) the products of re-esterification were extracted with chloroform and separated into methyl esters and alcohols using SiO_2-column chromatography. The alcohols were oxidized with CrO_3/acetic acid in cyclohexane in order to get the respective fatty acids which have been esterified as mentioned above. Determination of the double bond position of unsaturated fatty acids was carried out by mass spectrometry after oxidation with OsO_4 in pyridine/dioxane [17] and subsequent silylation with Trisil/N,O-bis-trimethylsilyl-trifluoroacetamide [18]. Identification of all components was achieved by gas chromatographic and mass spectrometric comparison with authentic reference substances. Gas chromatography was performed with 50 m glass capillaries (impregnation CPsil 5) at 200 °C column temperature by using a Perkin-Elmer instrument (type Sigma 2B) and an electronic integrator Spectra-Physics SP 4100-02. For gas chromatography/mass spectrometry combination the same type of column was used. Mass spectra were recorded with the Varian-MAT 112S instrument at 70 eV and 200 °C ion source temperature.

Results and Discussion

After thin-layer and column chromatography, the secretion of the anal sacs of *Civettictis civetta* showed to be a mixture of various lipids, the quantitative composition of which is given in Table I.

The quantity of the compound lipids (cholesterol esters, mono- and diester waxes, triglycerides) approximately equals that of the free constituents (free alcohols, cholesterol, free fatty acids). This holds true for the ratio free fatty acids/alcoholic constituents. The quantitative composition of the fatty acids after methanolysis of the monoester waxes and cholesterol esters which were not separated by SiO_2 column chromatography, and of the triglycerides is compared with the composition of the free fatty acids in Table II. The qualitative composition was found to be very similar in all fractions. Only the cholesterol esters are distinguished by the occurrence of more highly unsaturated acids. The only substituted acids found were $(\omega - 1)$ and $(\omega - 2)$-methylsubstituted tetradecanoic acids whereas highly volatile short-chain free fatty acids could not be detected.

Table I. Quantitative composition of the crude lipids of the secretion from the anal sacs of *Civettictis civetta*.

Lipid	mg	%
Cholesterol esters	20.6	16.1
Monoester waxes	28.5	22.3
Diester waxes	5.0	3.9
Triglycerides	7.3	5.7
Free alcohols	3.7	2.9
Cholesterol	29.8	23.3
Free fatty acids	32.9	25.8

The double bonds of the monounsaturated fatty acids were located in the $(\omega - 6)$ and $(\omega - 8)$ position, respectively as indicated by mass spectrometry of the TMS-ethers of the products from the OsO_4-oxidation. The diunsaturated fatty acids may be derived from the monounsaturated ones by further desaturation.

The qualitative and quantitative composition of the free alcohols as well as of the alcohols of the monoester waxes are very similar (Table 3). Concerning the diester waxes it could only be proved that saturated fatty acids and alcohols are involved in their formation.

As to the over all composition of the secretion, it is remarkable that similarily high quantities of wax esters, cholesterol, and cholesterol esters were found. The reason for this may lie in the specific histology of the anal sacs of *Civettictis*. Apart from the sebaceous glands (and the apocrine glands), it is most probable that the entire epithelial lining of the anal sacs actively produces secretion. Downing *et al.* [19] have pointed out that the composition of epidermal lipids differs from that of the sebaceous glands. The large proportion of the free fatty acids,

Table II. Quantitative composition of free fatty acids and fatty acids of monoester waxes, cholesterol esters, and triglycerides (% of the GC area).

	Monoester waxes/ Cholesterol esters	Triglycerides	Free fatty acids
Saturated unbranched fatty acids (total)	(40.2)	(56.8)	(66.1)
12:0	0.1	–	–
13:0	0.1	–	–
14:0	0.8	3.3	1.1
15:0	1.0	1.5	1.3
16:0	19.2	38.6	31.6
17:0	0.5	0.1	1.6
18:0	17.3	13.3	30.5
20:0	1.2	–	–
Saturated branched fatty acids (total)	(2.5)	(3.9)	(3.1)
12-C_{14}	0.9	1.0	0.7
13-C_{14}	1.6	2.9	2.4
Unsaturated fatty acids (total)	(57.3)	(39.3)	(30.8)
16:1 (7)[a]	1.2	3.9	0.9
16:1 (9)	1.2	2.3	0.8
18:1 (9)	22.1	14.3	19.4
18:1 (11)	16.9	8.9	9.7
20:1 (13)	0.5	–	–
18:2 (8, 11 + 9, 12)	5.4	9.9	–
20:2 (10, 13)	0.1	–	–
20:3	1.1	–	–
20:4	5.3	–	–
22:polyene	3.5	–	–

[a] C atom with double bond position.

Table III. Quantitative composition of monoester waxes and free alcoholic constituents (% of the GC area).

Alcohol	Monoester waxes	Free alcohols
14:0	0.7	0.9
15:0	2.6	1.3
16:0	33.1	32.2
17:0	5.1	3.3
18:0	57.0	48.2
18:1	1.5	14.1

compared to the small amount of triglycerides may indicate extensive bacterial hydrolysis of the sebum from the anal sacs. Remarkably, the secretion does not contain any volatile fatty acids. This is consistent with the present observations in that the freshly obtained secretion was nearly scentless. Although not too great a weight can be placed on these findings which are after all based on a single individual, they nevertheless hint that completely different conditions are found in *Herpestes* [6], *Vulpes* and *Panthera* [20]. Gorman *et al.* [6] reported in their study the occurrence of a series of short-chain saturated carboxylic acids (acetic, propionic, isobutyric, butyric, isovaleric, and valeric acid). Albone and Grönneberg [20] found highly volatile acids (C_2-C_6) in all secretions analysed and additionally indol in *Vulpes*.

Considering these findings, it remains difficult to answer the question of the functional significance of the anal sacs of *Civettictis*. According to the relatively complicated morphology of these organs it seems highly improbable that they are functionally accidental thus contradicting Ewer's opinion [14]. Since, however, little information on the biology of *Civettictis* is available the interpretation of the findings must be speculative. However, it seems conceivable that, apart from the perineal glands *Civettictis* uses the anal sacs for scent marking, adding its secretion to the faeces. Scent marking by anal sac secretion would have another meaning than that of the secretion of the perineal glands. Ewer and Wemmer [3] observed that always the same spots were used for defaecation by their captive specimens and that the faeces had a significant

scent. Like Kleiman-Eisenberg [21], they too pointed at the biological importance of dung heaps for forwarding information. It would make sense indeed, if such dung heaps have a long lasting odour as was interestingly found in *Genetta* by Wemmer [7]. Long lasting scent effects cannot be produced by highly volatile substances. However, it may well be that the secretions of the anal sacs of *Civettictis* meet such requirements, provided after delivery together with the faeces, that they are decomposed by bacteria. In this connection it is not unlikely that the unusual positions of the double bonds of some unsaturated fatty acids are of certain importance.

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