

MAMMALIAN EXOCRINE SECRETIONS. XVII: CHEMICAL  
CHARACTERIZATION OF PREORBITAL SECRETION  
OF MALE SUNI, *Neotragus moschatus*

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**Abstract**—Gas chromatographic and gas chromatographic–mass spectrometric techniques were employed to identify 83 compounds, including alkanes, alkenes, aldehydes, 2-methylalkanes, carboxylic acids, 1-alkyl formates and alken-1-yl formates, benzoic acid, and cholesterol, in the preorbital secretion of the male suni, *Neotragus moschatus*. Dimethyl disulfide derivatization and lithium aluminum hydride reduction were used to determine the position of double bonds and to confirm the identity of the functional groups in some of the constituents of the secretion.

**Key Words**—Suni, *Neotragus moschatus*, semiochemicals, pheromones.

INTRODUCTION

Extensive research has been done on the role of the secretions of the preorbital glands of hoofed mammals or ungulates of the order Artiodactyla in their rutting and territorial behavior (e.g., Langguth and Jackson, 1980; Mossing and Damber, 1981; Frey and Hofmann, 1997; Marmazinskaya, 1997; Roberts, 1997, 1998; Arcese, 1999; Brashares and Arcese, 1999; Lawson et al., 2000, 2001), but in spite of the biological importance attributed to them, little is known about the chemical composition of preorbital secretions. Andersson (1979) has identified two ketones in the preorbital pouch secretion of the reindeer, *Rangifer t. tarandus*. Several heavy constituents such as cholesterol, lanosterol, fatty acids, and triglycerides were subsequently identified in this secretion (Sokolov et al., 1977). Cholesterol, benzaldehyde, and a homologous series of saturated  $\gamma$ -lactones have been identified

in the preorbital secretion of the muskox, *Ovibos moschatus* (Flood et al., 1989). The preorbital secretions of the bontebok, *Damaliscus dorcas dorcas*, and blesbok, *D. d. phillipsi*, contain, among other compounds, some unbranched primary alcohols, a few short-chain saturated and several long-chain saturated and unsaturated unbranched carboxylic acids and aldehydes, three long-chain  $\delta$ -lactones, benzoic acid, 4-hydroxybenzoic acid, 4-hydroxybenzaldehyde, 2-heptanone, piperitone, dimethylsulfone, isopropyl tetradecanoate, isopropyl hexadecanoate, hexadecyl acetate, cholesterol,  $\alpha$ -tocopherol, and squalene (Burger et al., 1999a).

With the exception of the female oribi, *Ourebia ourebi*, which does not produce a preorbital secretion (Mo et al., 1995), and the preorbital secretion of female klipspringer, *Oreotragus oreotragus*, which has not yet been investigated, the males and females of the other members of the subfamily Antilopinae produce qualitatively identical preorbital secretions. In sharp contrast, exploratory gas chromatographic–mass spectrometric (GC-MS) analyses of the preorbital secretions of suni, *Neotragus moschatus*, a dwarf antelope of the tribe Neotragini, revealed prominent qualitative and quantitative differences in the secretions of male and female animals. The suni was, therefore, selected for further study of its preorbital secretions in a continuing investigation of the role of preorbital secretions in the territorial behavior of South African antelope species.

#### METHODS AND MATERIALS

*General.* All Pyrex glassware was thoroughly cleaned with water and organic solvents and then heated at 500°C in an annealing oven to remove any trace of organic contaminants. Syringes and other apparatus that could not be heated to 500°C were cleaned with dichloromethane (Merck, Residue Analysis Grade). Preorbital secretions were extracted and the extracts diluted, where necessary, with this solvent.

*Collection and Sample Preparation.* Preorbital gland secretions were collected with a tubular PTFE scoop from male suni kept in pens (10 × 10 m) at the Tygerberg Zoo, Cape Town, South Africa. The scoop, with an inside diameter of 5 mm, was furnished with a PTFE plunger with which the collected material could be ejected into a 1-ml Reacti-Vial. Samples were taken at different times of the year as needed from six males bred from two unrelated lineages. To avoid the risk of losing animals, males were not trapped in the presence of pregnant females.

The organic constituents were extracted from the mucoid secretion by stirring the collected material with an appropriate quantity of dichloromethane, using a thin (ca. 1 mm diam.) glass rod, centrifuging the resulting suspension at 3500 rpm for 15 min, and removing the organic fraction from underneath the supernatant mucoid layer with a 100- $\mu$ l syringe. In a typical sample preparation, 108 mg of secretion was collected from the glands of a male and extracted with 120  $\mu$ l of

dichloromethane to give 97  $\mu\text{l}$  of extract. This extract was used without further concentration for quantitative determination of the volatile organic constituents. However, to avoid having to concentrate extracts for GC-MS analysis with the concomitant loss of volatiles, the organic material was mostly extracted with the smallest possible volume of solvent that still gave a separable solvent layer. Extracts were transferred to clean Reacti-Vials and stored at  $-30^{\circ}\text{C}$  until used for analysis.

*Analytical Methods.* Instrumentation for the identification of the volatiles has been described in detail by Burger et al. (1996). GC and GC-MS analyses were done with the following capillary columns: (1) 40-m  $\times$  0.3-mm glass column coated with PS-089 (polarity equivalent to that of SE-52) at a film thickness of 0.25  $\mu\text{m}$ , and (2) 30-m  $\times$  0.25-mm glass column coated with OV-240 at a film thickness of 0.25  $\mu\text{m}$ . Helium was employed as carrier gas at a linear velocity of 28.6 cm/sec at  $40^{\circ}\text{C}$ . Samples were injected at an injector temperature of  $220^{\circ}\text{C}$ , thermally focused on the column at  $30^{\circ}\text{C}$  and analyzed using a temperature program of  $2^{\circ}\text{C}/\text{min}$  from  $40^{\circ}\text{C}$  to  $270^{\circ}\text{C}$  and holding the temperature at  $270^{\circ}\text{C}$  for 80 min. The flame ionization detector was operated at  $280^{\circ}\text{C}$ . Quantitative GC analyses were done with the same instrument and the PS-089 column, and data acquisition with Borwin Intuitive Chromatography Software (JMBS Developments, 38600 Fontaine, France) using hexadecanoic acid as external standard.

Low-resolution electron impact mass spectra (EI-MS) were obtained at 70 eV on a Carlo Erba QMD 1000 GC-MS instrument by using the columns and temperature program specified above. An ion source temperature of  $100^{\circ}\text{C}$  was used. Chemical ionization mass spectra, with methane as reactant gas [CI( $\text{CH}_4$ )-MS], were obtained on an AMD 604 double-focusing mass spectrometer at a resolution of 1000 and a mass range of 100–500 atomic mass units.

*Dimethyl Disulfide Derivatization.* DMDS derivatization was carried out according to the method of Vincenti et al. (1987). A dichloromethane extract (18  $\mu\text{l}$ ) of the secretion was concentrated in a 1-ml Reacti-Vial with a slow stream of purified (activated charcoal) nitrogen. Residual dichloromethane was removed by the addition and slow evaporation of carbon disulfide 50  $\mu\text{l}$ . This process was repeated three times. The residual material was dissolved in 50  $\mu\text{l}$  carbon disulfide, and 5  $\mu\text{l}$  of an iodine solution (60 mg iodine in 1 ml diethylether) as well as 50  $\mu\text{l}$  dimethyl disulfide were added. The screw-capped vial was sealed using a PTFE-lined septum and left in the oven of a gas chromatograph at  $60^{\circ}\text{C}$  for 40 hr. The reaction was quenched with an aqueous solution of sodium thiosulfate (5%), and the DMDS derivatives were isolated by centrifuging the reaction mixture for a few minutes at 2000 rpm. The organic layer was transferred to a clean Reacti-Vial with a 100- $\mu\text{l}$  syringe and concentrated to 5  $\mu\text{l}$  for GC-MS analysis.

*Lithium Aluminium Hydride Reduction.* The organic constituents of the secretion were subjected to lithium aluminum hydride reduction to confirm the presence of reducible functional groups. A 7- $\mu\text{l}$  aliquot of the extract was evaporated to dryness in a stream of nitrogen. The residual material was redissolved

twice in diethyl ether, evaporated to eliminate traces of dichloromethane, and then dissolved in diethyl ether (30  $\mu$ l) and treated with a saturated solution of LiAlH<sub>4</sub> in ether (20  $\mu$ l). The Reacti-Vial was closed using a PTFE-lined septum and heated for 5 min at 40°C, after which the reaction mixture was cooled and treated with cold water (30  $\mu$ l), centrifuged, the organic layer transferred to a clean Reacti-Vial, and the resulting solution concentrated for GC-MS analysis.

*Reference Compounds.* Some of the compounds identified in the preorbital secretion of the male suni were available from previous research projects in this series, while others were obtained commercially. Unsaturated alcohols were purchased from Pherobank (Wageningen, The Netherlands). The following compounds were synthesized.

*2-Methylcosane.* A mixture of 1-bromohexadecane (2.78 g, 0.01 mol), 1-bromo-3-methylbutane (1.51 g, 0.01 mol), and sodium (0.92 g, 0.04 mol) was refluxed under argon until the Wurtz condensation started. The reaction went to completion within 3 hr. The unreacted sodium was destroyed with ethanol, whereafter water (5 ml) was added to the reaction mixture and the organic material extracted with hexane. According to GC-MS analysis, the extract contained 2,7-dimethyloctane (26%), 1-hexadecene (49%), 1-hexadecanol (6%), 2-methylcosane (18%), and dotriacontane (1%). EI-MS of 2-methylcosane: *m/z* 43 (85), 57 (100), 71 (66), 85 (45), 99 (18), 113 (8), 127 (6), 141 (5), 155 (4), and 253 (4%).

*1-Alkyl Formates.* A mixture of the primary alcohols, 1-butanol, 1-pentanol, 1-hexanol, 1-heptanol, 1-octanol, 1-nonanol, 1-decanol, 1-undecanol, 1-dodecanol, 1-tetradecanol, 1-hexadecanol, 1-octadecanol, 1-icosanol, 1-docosanol (15 mg each), and formic acid (420  $\mu$ l) was heated overnight at 80°C and the products extracted with hexane for comparison with the constituents of the secretion.

*Alken-1-yl Formates.* A mixture of (*Z*)- and (*E*)-6-tridecen-1-yl formate, (*Z*)- and (*E*)-8-tetradecen-1-yl formate, and (*Z*)- and (*E*)-8-hexadecen-1-yl formate was prepared by heating the corresponding alkenols with formic acid and extracting the products from the reaction mixture as described above for the preparation of the saturated formates.

## RESULTS AND DISCUSSION

The preorbital secretions of male and female suni differ both qualitatively and quantitatively, although they do contain a few constituents that are common to both. Male glands appear to be more productive than those of females, and the male secretion is also more complex. A series of related compounds forming the bulk of the female secretion could not be identified despite the availability of extensive 600-MHz NMR, HR-MS, and other physical data. Therefore, only the male secretion will be discussed here.

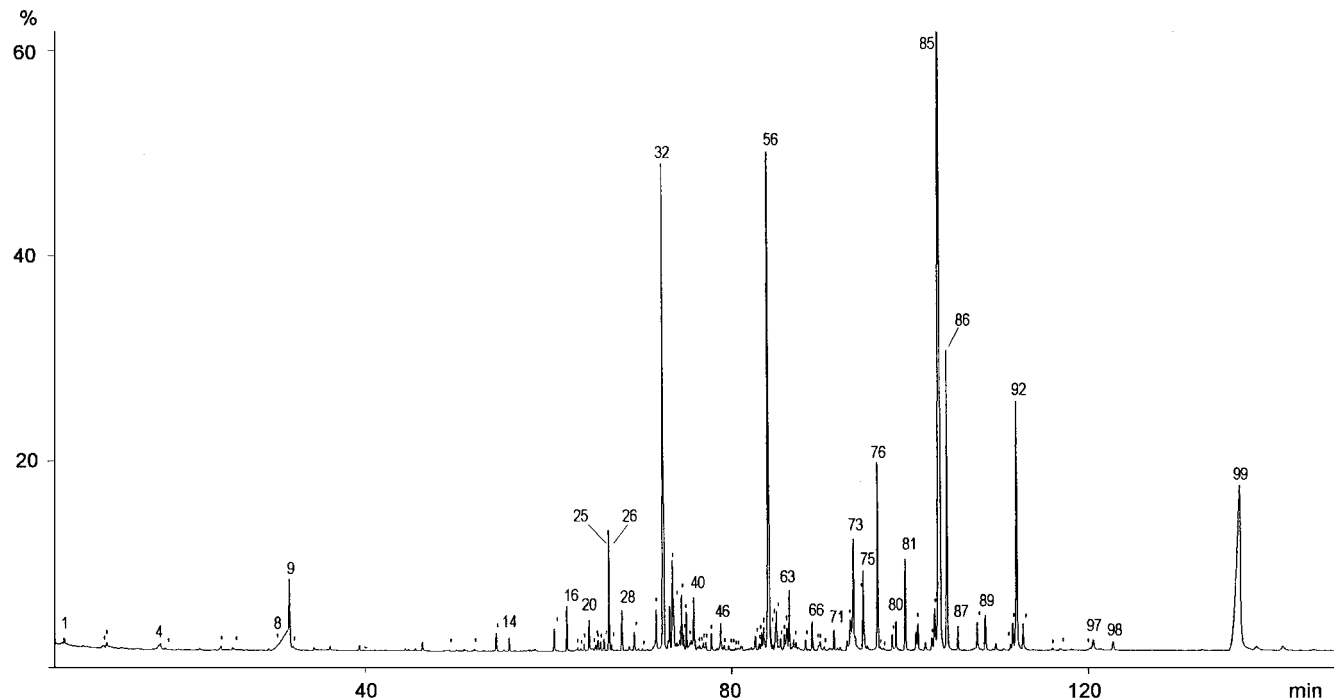


FIG. 1. Total ion chromatogram of an extract of the preorbital secretion of male suni, *Neotragus moschatus*. Glass capillary column coated with a 0.25- $\mu$ m film of the apolar stationary phase PS-089, programmed at 2°C/min from 40°C to 270°C (hold).

A typical total ion chromatogram of the male secretion is shown in Figure 1. At the level of sensitivity at which the qualitative analyses were done, secretions from individual males were identical. Constituents from the male were tentatively identified by comparison of their mass spectra with mass spectral data in the NBS and Wiley mass spectral libraries. The identification of many compounds could be confirmed by coinjection of commercially available reference compounds or synthesized material. The molecular mass of some of the compounds and, therefore, their chain lengths were confirmed by  $\text{CI}(\text{CH}_4)$ -MS data.

There are two homologous series of alkanes present in the secretion. The first consists of long-chain, unbranched alkanes, the structures of which could easily be confirmed by GC coelution with reference compounds, and the second series contains saturated hydrocarbons with the typical EI mass spectra of isoalkanes. 2-Methylcosane was synthesized as a model compound to confirm interpretation of the mass spectral data. A series of unbranched, long-chain fatty acids, compounds that appear to be almost ubiquitous in the exocrine secretions of various antelope species, were also found in this secretion. These and the other compounds identified in the secretion are listed in Table 1.

Dimethyl disulfide derivatization and GC-MS analysis of the DMDS derivatives were used to determine the presence and position of double bonds in the unsaturated constituents of the secretion (Vincenti et al., 1987). The interpretation of the mass spectra of the DMDS derivatives was discussed in detail previously (Burger et al., 1996). Due to the complexity of the male secretion and extensive coelution of its constituents, almost none of the peaks in the chromatogram in Figure 1 represent pure compounds. With the exception of a few alkenyl formates identified using the PS-089 column, it was impossible to correlate the unsaturated constituents with their DMDS derivatives using the apolar PS-089 column. However, an OV-240 capillary column gave better separation of the large number of alkenes, saturated and unsaturated formic acid esters, and DMDS derivatives of the unsaturated compounds than the PS-089 column. The unsaturated compounds are listed in Table 1 in the order in which they eluted from the OV-240 column. Although these compounds do not necessarily elute in the same order from the less polar PS-089 column, the numbers are used in Figure 1 to indicate approximately where the different homologous groups of unsaturated compounds elute in the total ion chromatogram. In a few cases, DMDS derivatization showed both the *E* and *Z* isomers of the unsaturated alkenes and formates present in the secretion. It was, therefore, possible to assign *E* or *Z* configuration to the double-bond isomers based on the GC elution order of these compounds and their DMDS derivatives. The double-bond configuration of a few of the unsaturated constituents was also determined by GC retention time comparison with authentic samples. The elucidation of the stereochemistry of all unsaturated and chiral constituents of the secretion was not attempted. The stereochemistry of some of the unsaturated and

TABLE 1. COMPOUNDS IDENTIFIED IN PREORBITAL SECRETION OF MALE SUNI

No. in Fig.1 (PS-089 column)	Compound (arranged according to compound type)	Analytical method <sup>a</sup>	Quantity ( $\mu\text{g}/\text{animal}$ )
13	Pentadecane	a,b,d	46
25	Heptadecane	a,b,d	137
45	Nonadecane	a,b	45
65	Henicosane	a,b,d	23
79	Tricosane	a,b,d	23
12	2-Methyltetradecane	a,d	1
20	2-Methylhexadecane	a	76
30	2-Methylheptadecane	a,d	5
40	2-Methyloctadecane	a,d	169
63	2-Methylcosane	a,b,d	208
71	2-Methylhenicosane	a,d	59
76	2-Methyldocosane	a,d	300
82	2-Methyltricosane	a	48
87	2-Methyltetracosane	a,d	46
9	Impurity	a	
17	(Z)-6-Heptadecene	a,e,g	7
18	5-Heptadecene	a,e,g	6
19	(Z)-4-Heptadecene	a,e,g	15
21	7-Heptadecene	a,e,g	9
22	(E)-6-Heptadecene	a,e,g	16
23	(E)-4-Heptadecene	a,e,g	13
24	Unidentified heptadecene	a,h	21
35	(Z)-7-Nonadecene	a,e,g	16
36	6-Nonadecene	a,e,g	133
37	5-Nonadecene	a,e,g	31
38	9-Nonadecene	a,e,g	81
39	(E)-7-Nonadecene	a,e,g	11
41	4-Nonadecene	a,e,g	9
42	Unidentified nonadecene	a,h	5
43	Unidentified nonadecene	a,h	11
44	Unidentified nonadecene	a,h	14
48	5-Icosene	a,e,g	3
49	Unidentified icosene	a,h	3
50	Unidentified icosene	a,h	4
58	8-Henicosene	a,e,g	117
59	(Z)-9-Henicosene	a,e,g	103
60	10-Henicosene	a,e,g	31
61	7-Henicosene	a,e,g	68
62	6-Henicosene	a,e,g	141
64	(E)-9-Henicosene	a,e,g	23
68	9-Docosene	a,e,g	17
69	10-Docosene	a,e,g	14
74	9-Tricosene	a,e,g	3
77	10-Tricosene	a,e,g	5
78	11-Tricosene	a,e,g	5

TABLE 1. CONTINUED

No. in Fig.1 (PS-089 column)	Compound (arranged according to compound type)	Analytical method <sup>a</sup>	Quantity ( $\mu\text{g}/\text{animal}$ )
1	Hexanal	a,b,f	2
3	Heptanal	a,b,f	2
5	Octanal	a,b,f	0.5
7	Nonanal	a,b,f	1
2	Pentanoic acid	a,b,f	1
4	Hexanoic acid	a,b,f	3
6	Heptanoic acid	a,b,f	1
8	Benzoic acid	a,b,f	23
10	Octanoic acid	a,b,f	1
51	Hexadecanoic acid	a,b,f	7
70	Octadecanoic acid	a,b,f	26
11	1-Undecyl formate	a,b,c,d,f	5
14	1-Dodecyl formate	a,b,c,d,f	34
16	1-Tridecyl formate	a,b,c,d,f	107
28	1-Tetradecyl formate	a,b,c,d,f	103
34	1-Pentadecyl formate	a,f	73
47	1-Hexadecyl formate	a,b,c,f	3
57	1-Heptadecyl formate	a,d,f	25
67	1-Octadecyl formate	a,b,c,f	14
75	1-Nonadecyl formate	a,d,f	195
81	1-Icosyl formate	a,b,c,d,f	273
86	1-Henicosyl formate	a,d,f	762
89	1-Docosyl formate	a,b,c,d,f	162
93	1-Tricosyl formate	a,d,f	130
95	1-Tetracosyl formate	a,f	30
15	(Z)-6-Tridecen-1-yl formate	a,c,e,f,g	57
26	7-Tetradecen-1-yl formate	a,e,f,g	42
27	(Z)-8-Tetradecen-1-yl formate	a,c,e,f,g	19
32	8-Pentadecen-1-yl formate	a,d,e,f	782
53	8-Heptadecen-1-yl formate	a,e,f,g	14
54	10-Heptadecen-1-yl formate	a,d,e,f	4
72	10-Nonadecen-1-yl formate	a,e,f,g	60
73	12-Nonadecen-1-yl formate	a,d,e,f	171
80	13-Icosen-1-yl formate	a,e,f,g	107
83	12-Henicosen-1-yl formate	a,e,f,g	72
84	16-Henicosen-1-yl formate	a,e,f,g	206
85	14-Henicosen-1-yl formate	a,d,e,f	2319
88	15-Docosen-1-yl formate	a,e,f,g	142
90	14-Tricosen-1-yl formate	a,e,f,g	53
91	18-Tricosen-1-yl formate	a,e,f,g	137
92	16-Tricosen-1-yl formate	a,d,e,f	761
94	17-Tetracosen-1-yl formate	a,e,f,g	27
96	16-Pentacosen-1-yl formate	a,e,f,g	36
97	18-Pentacosen-1-yl formate	a,e,f,g	106
98	Unidentified steroid	a,b,f	2



TABLE 1. CONTINUED

No. in Fig.1 (PS-089 column)	Compound (arranged according to compound type)	Analytical method <sup>a</sup>	Quantity ( $\mu\text{g}/\text{animal}$ )
99	Cholesterol	a,b,f	1848
29	Unidentified		149
31	Unidentified		96
33	Unidentified		197
46	Unidentified		69
52	Unidentified		124
55	Unidentified		3
56	Unidentified		862
66	Unidentified		46

<sup>a</sup>a: Low-resolution GC-MS; b: retention-time comparison using PS-089 column; c: retention-time comparison using OV-240 column; d:  $\text{Cl}(\text{CH}_3)\text{-MS}$ ; e: DMDS derivatization; f: reduction with  $\text{LiAlH}_4$ ; g: unsaturated compounds listed in the order in which they are eluted from the OV-240 column, elution order interchangeable on the PS-089 column within each group of homologs; h: not observed with OV-240 column.

chiral constituents of the exocrine secretions of various South African antelope species will be dealt with in a future publication.

It is often difficult to differentiate between saturated and unsaturated long-chain alcohols, formates, and alkenes, especially if they are present in such small quantities that it is difficult to obtain pure spectra from a GC-MS analysis. These spectra often also lack diagnostic ions in the higher mass ranges. Furthermore, the preparative GC isolation of constituents is not feasible if they are present in low concentrations and only a limited quantity of secretion is available. Valuable diagnostic information was, however, obtained by lithium aluminum hydride reduction of the whole extract of the secretion of the male, which left some constituents intact, whereas carbonyl compounds, esters, etc., were reduced to compounds that coeluted with the reduction products of other reducible constituents. Information on the contribution of this experiment to the identification of some of the constituents of the secretion is included in Table 1.

Quantitative extraction of the volatile organic material from the secretion was not possible with small quantities of solvent. The widely varying quantities of secretion collected from individual males furthermore contained considerable proportions of water, as well as heavy material that does not pass through the GC column. It is, therefore, impossible to determine the average quantitative composition of the preorbital secretions of males with even reasonable accuracy. The quantitative results in Table 1 are nevertheless included to give at least an approximate indication of the quantities of most of the compounds present in a relatively large sample of secretion collected from a single male.

The suni is the fifth of several small South African antelope species belonging to the tribe Neotragini of which the chemical composition of the preorbital secretion

TABLE 2. COMPOUND TYPES IDENTIFIED IN PREORBITAL SECRETIONS OF ANTELOPE OF TRIBE NEOTRAGINI

Compound types	Carbon numbers of compounds and other structural information				
	Male and female grysbok, <i>Raphicerus melanotis</i>	Male and female steenbok, <i>R. campestris</i>	Male oribi, <i>Ourebia orebi</i>	Male suni, <i>Neotragus moschatus</i>	Male klipspringer, <i>Oreotragus oreotragus</i>
<i>n</i> -Alkanes		8–10,12		15,17,19,21,23	
Isoalkanes				15,17,18,19,21–25	
Alkenes				17,19–23 (30) <sup>a</sup>	
Alkan-1-ols	11–15	8–16,20,23–27	9,10		
Alken-1-ols	12–17,19,21,23 (13) <sup>a</sup>	7,10–15 (9) <sup>a</sup>	10,12–14 (4) <sup>a</sup>		
Alkadien-1-ols	13–15,17 (4) <sup>a</sup>	17 (1) <sup>a</sup>	13–16 (4) <sup>a</sup>		
Alkan-1-yl formates	11–25	11–13,20–28	8–16	11–24	
Alken-1-yl formates	13–25 (18) <sup>a</sup>	11–15 (6) <sup>a</sup>	9–15 (8) <sup>a</sup>	13–15,17,19–25 (19) <sup>a</sup>	
Alkadien-1-yl formates	14,15,17,19,21 (5) <sup>a</sup>		12–18 (7) <sup>a</sup>		
Alkan-1-yl acetates		13,15,16	8–17		
Alken-1-yl acetates		13,15,19,21,23,24 (6) <sup>a</sup>	10–17 (10) <sup>a</sup>		
Alkadien-1-yl acetates			13–18 (6) <sup>a</sup>		
Alkanals	12,13	6,7,9	9–13	6–9	
Alkenals	13 (1) <sup>a</sup>		11–14 (4) <sup>a</sup>		
Alkadienals		10 (2) <sup>a</sup>	13–16 (4) <sup>a</sup>		
Cycloalkanones		16–21			

Alkanoic acids	14–18,20	4,8,10,12,14–16,18,20	16–18	5–8,16,18	
Alkenoic acids		12–14,16,18 (5) <sup>a</sup>			
Alkadienoic acids		18 (1) <sup>a</sup>			
Alkan-4-olides ( $\gamma$ -lactones)	15–18,20				
Alkan-5-olides ( $\delta$ -lactones)	16				
1-Hydroxyalk-2-yl acetates		18,20–22			
2-Hydroxyalk-1-yl acetates	18,20	17,18,20–23			
1-Hydroxyalk-2-yl butanoates		18,20,22			
2-Hydroxyalk-1-yl butanoates		14,16,18,20,22			
Miscellaneous	Heptadec-1-yl methyl sulfide	Isopropyl tetradecanoate	13-Methyl-(Z)-8-pentadecen-1-yl formate	Benzoic acid	3-Pentanone
	Methyl nonadec-1-yl sulfide	Isopropyl hexadecanoate		Cholesterol	4-Methyl-2-pentanone
		2-Methylbutanoic acid			
		3-Methylbutanoic acid			
		5-Methyl-3-hexanol			5-Methyl-3-hexanone
		Limonene			4-Methyl-3-hexanone
		Squalene			Ethyl propanoate
		Cholesterol			2-Methylpropyl acetate
		$\alpha$ -Tocopherol			Ethyl-3-methyl butanoate
					2-Methylpropyl propanoate

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<sup>a</sup>Total number of double-bond positional and configurational isomers.

has been investigated. Of these antelope, the male klipspringer, *Oreotragus oreotragus*, (Burger et al., 1997) is unique in that the male preorbital secretion contains only eight short-chain, volatile organic compounds: four ketones and four esters. The female preorbital secretion of the klipspringer has so far not been investigated. As mentioned in the introduction, female oribi, *Ourebia orebi* (Mo et al., 1995), do not produce any preorbital secretion, although they possess an external structure that resembles that of the preorbital gland of the male. The preorbital secretions of male and female grysbok, *Raphicerus melanotis*, (Burger et al., 1996) and steenbok, *R. campestris*, (Burger et al., 1999b) have also been reasonably well characterized. Male and female secretions of the two *Raphicerus* species are qualitatively identical. Perhaps the most remarkable feature of members of this tribe is the large number of long-chain saturated and unsaturated formic acid esters present in all secretions investigated so far, with the exception of the klipspringer's preorbital secretion. The results from members of the tribe Neotragini are summarized in Table 2. Due to the large number of double-bond positional and configurational isomers present in some members of this tribe, it is impossible to include all relevant information in Table 2. However, there does not seem to be any indication that the position of double bonds in the unsaturated constituents and their stereochemistry could play a part in the territorial behavior of these animals.

One aspect that merits consideration in future research is the possibility that some constituents of the secretions are not secreted by the preorbital glands but are produced by different strains of the same bacterium or by different bacteria present in the glandular structures of the animals. This is supported by the observation that the secretions are more complex in the members of the tribe that produce secretion only very slowly, whereas the secretion of the male klipspringer, produced at a rate of about 5 mg/min, contains only eight simple compounds. In this case, microbial action probably cannot add to the secretion's complexity before it is deposited as a territorial mark and dries out.

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