# MAMMALIAN EXOCRINE SECRETIONS: X. CONSTITUENTS OF PREORBITAL SECRETION OF GRYSBOK, Raphicerus melanotis

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Abstract—Using electron impact and chemical ionization mass spectrometry with methane and nitric oxide as reactant gases in conjunction with mass spectral analysis of the dimethyl disulfide derivatives of unsaturated constituents, 51 compounds have been identified in addition to the compounds previously found in the preorbital secretion of the grysbok *Raphicerus melanotis*. The compounds identified are saturated, monounsaturated, and diunsaturated alcohols and formates, saturated and monounsaturated aldehydes, saturated carboxylic acids,  $\gamma$ - and  $\delta$ -lactones, alkyl methyl sulfides, and  $\beta$ -hydroxyalkyl acetates. All of these compounds have unbranched structures. The constituent previously incorrectly identified as (Z)-5-tetradecen-1-ol, was found to consist of the coeluting (Z)-7- and (Z)-8-tetradecenols.

Key Words—Raphicerus melanotis, mammalian semiochemicals, mammalian pheromones, exocrine secretion, preorbital secretion, double bond localization, mass spectrometry.

#### INTRODUCTION

The Cape grysbok, *Raphicerus melanotis*, is a small, squat antelope characterized by rufous brown upperparts abundantly flecked with white hairs and underparts with a lighter brown color. The incidence of white hairs diminishes down the sides and legs. The tail is short and the grey-brown ears are proportionately large with white hairs on the inside. The average shoulder height is about 54 cm and the average mass is about 10 kg. Only rams have short, smooth, slightly

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back-angled horns with an average length of about 8 cm (Stuart and Stuart, 1990). The rufous brown upperparts flecked with white distinguish it from the steenbok, as do the brown underparts. The Cape grysbok is similar to Sharp's grysbok, *Raphicerus sharpei*, in appearance, but their distribution ranges do not overlap (Stuart and Stuart, 1990).

The distribution of the grysbok is restricted to a narrow belt along the southwestern and southern Cape coast and adjacent interior of South Africa. An inhabitant of relatively thick scrub-bush, it is almost entirely restricted to the "fynbos" vegetation of the Cape. It is mainly a nocturnal animal but is active in the early morning and late afternoon if not disturbed, or on overcast and cool days. Grysbok are usually single except when mating or when ewes are tending lambs. Although lambs may be dropped at any time of the year, most are born from September to December. A single lamb is born after a gestation period of approximately 180 days. The grysbok is considered to be a nuisance in vineyards as it grazes on the young grapes and terminal buds.

The grysbok belongs to the order Artiodactyla, the family Bovidae and the subfamily Antilopinae. Members of this subfamily, also known as dwarf antelope, vary considerably in appearance and habitat. The pygmy antelope, Neotragus batesi, for instance, inhabits the dense forests of Central Africa, whereas the oribi, Ourebia ourebi, is found on open grass plains near water. The klipspringer, Oreotragus oreotragus, prefers mountainous territory and the dik-dik, Madoqua kirkii, is found in arid bushveld regions. The only similarities between members of this subfamily are their size and their well-developed exocrine glands, especially the preorbital glands, which are used for territorial marking (Macdonald, 1984). Territoriality is important to these animals as it is essential for them to have the necessary knowledge regarding food supplies in their environment and to eliminate any competition with regard to their food supply. Feeding is an important factor because their metabolic processes are rapid as a result of their small body size. Consequently, in relation to their body size, they need more nutritious food than larger animals (Macdonald, 1984). As the grysbok is found in areas with dense vegetation, olfactory communication is expected to be an important means of communication. Both sexes possess active preorbital glands, the secretions of which consist of a black aqueous emulsion of organic compounds. Grysbok rams are solitary and territorial and mark territories with the preorbital secretion (Novellie et al., 1984). Although no qualitative and only small, probably insignificant, differences were found between the male and female secretions (Le Roux, 1980), marking activity is only rarely observed in females.

In a previous study (Le Roux, 1980; Burger et al., 1981a) 34 of the constituents of the preorbital secretion of the grysbok were identified. Due to the uninformative mass spectra of long-chain, unbranched compounds, only a limited number of these constituents were identified solely by means of mass spectrometry. Some of the major representative constituents were therefore isolated for NMR analysis using preparative gas chromatography. This entailed the collection of 1000 gland equivalents of the secretion from five animals over many months. In spite of an enormous investment in time, only 18 constituents of the secretion were isolated in sufficient quantities for <sup>1</sup>H NMR analysis. Of five of these constituents <sup>13</sup>C NMR spectra could be obtained. The position of the double bonds in some of these compounds was determined by microozonolysis. The Z configuration was assigned to unsaturated compounds on the available <sup>1</sup>H and <sup>13</sup>C NMR data.

The availability of more efficient capillary columns, and new methods for the determination of the position of double bonds in long-chain compounds, led to the decision to reinvestigate the preorbital secretion of the grysbok. In this communication we report the confirmation of the structures of 27 of the compounds identified in the previous investigation and the identification of another 51 constituents of the secretion.

#### METHODS AND MATERIALS

General. All Pyrex glassware used in the handling of biological material and extracts, as well as in the preparation of reference compounds, was heated to 500°C in an annealing oven to remove any traces of organic material. Dichloromethane (Merck, Residue Analysis Grade) was analyzed gas chromatographically and found to be pure enough for extraction purposes when used in small quantities. Syringes, stainless-steel needles, etc., were cleaned by rinsing with this solvent.

Analytical Methods. Gas chromatographic (GC) analyses were carried out with Carlo Erba 4200 and 5300 gas chromatographs equipped with flame ionization detectors, Grob split-splitless injectors, and glass columns coated by the Laboratory for Ecological Chemistry with a 0.25-mm film of the apolar stationary phases, PS-089-OH, a silanol-terminated, 95%-dimethyl-5%-diphenylsiloxane copolymer, and OV-1701-OH. Helium was used as carrier gas at a linear velocity of 28.6 cm/sec at 40°C. The flame ionization detector was operated at 280°C and the injector was normally used at 220°C. In analyses of material dissolved in dichloromethane, the samples were injected in the split mode, and the volatiles entering the column were thermally focused at ca. 30°C, and subsequently analyzed using a temperature program of 2°C/min from 40°C to 260°C (hold). In analyses in which solventless sample introduction was used, cryotrapping with Dry Ice was employed. Quantification was done with Barspec software and a 386 personal computer.

Electron impact (EI) mass spectra were recorded at 70 eV on a Carlo Erba QMD 1000 gas chromatograph-mass spectrometer (GC-MS system), using the columns and conditions described above. The QMD 1000 consists of a Carlo Erba 5300 gas chromatograph with a line-of-sight interface to a VG Analytical Trio 1 quadrupole mass spectrometer. An interface temperature of 250°C was used. The ion source temperature was set at 200°C and the pressure in the source housing was ca.  $2 \times 10^{-5}$  torr at a column temperature of 40°C, decreasing to ca.  $1 \times 10^{-5}$  torr towards the end of the temperature program.

Chemical ionization (CI) mass spectra were obtained with methane and nitric oxide as reactant gases. With methane as reactant gas, spectra were recorded at 75 eV and a source temperature of  $150^{\circ}$ C. The flow of methane into the ion volume of the source was regulated with a fine metering valve to obtain equally abundant m/z 17 and 29 plasma ions. With NO as reactant gas, an iridium filament coated with thorium oxide was used. Spectra were recorded at 75 eV and a source temperature of ca. 80°C. The source pressure was adjusted to obtain a maximum abundance of the m/z 60 plasma ion. With isobutane as reactant gas, optimal tuning was reached by adjusting the source pressure to produce the m/z 39, 43, and 57 plasma ions in a ratio of 1:4:6. All three reactant gases required a source housing pressure of ca.  $8 \times 10^{-5}$  torr for optimal tuning.

Sample Collection and Preparation. Preorbital secretion was collected with a tubular PTFE scoop from animals kept in pens ( $10 \times 10$  m) at the Tygerberg Zoo. 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 300- $\mu$ l or 1-ml Reacti-Vial.

The organic volatiles were extracted from the secretion by stirring collected material with dichloromethane (ca. 100  $\mu$ l) using a thin (0.5 mm) glass rod to produce a homogeneous suspension that was centrifuged at 2000–3000 rpm for about 15 min to separate solid material, the dichloromethane extract, and a small quantity of water and mucus. The dichloromethane extract was removed from underneath the supernatant water and mucus layer with a 100- $\mu$ l syringe, transferred to a Reacti-Vial, and stored at  $-30^{\circ}$ C. Most extracts could be used without subsequent evaporation of the solvent. If more concentrated solutions were required, the solvent was removed in a slow stream of purified (activated charcoal) nitrogen.

Dimethyl disulfide (DMDS) derivatization of the organic material extracted from the secretion was carried out according to the method described by Vincenti et al. (1987). A 10- $\mu$ l aliquot of a dichloromethane extract of the preorbital secretion was concentrated in a 1-ml Reacti-Vial using a slow stream of purified nitrogen, the residual material dissolved in dimethyl disulfide (50  $\mu$ l), and treated with a solution of iodine (300  $\mu$ g) in carbon disulfide (50  $\mu$ l). The Reacti-Vial was sealed using a PTFE-faced rubber septum and the reaction mixture left at 60°C for 40 hr in the oven of a gas chromatograph. The reaction was quenched with aqueous sodium thiosulfate solution (5%). The organic and aqueous layers were separated by centrifuging for a few minutes at 2000 rpm after which the organic layer was transferred to another Reacti-Vial. The solution was concentrated to 5  $\mu$ l for GC-MS analysis.

*Reference Compounds.* Some of the compounds identified in the preorbital secretion of the grysbok are commercially available, while others were available from previous research projects in this series. The following compounds were synthesized during the present investigation.

*Heptadecanyl formate* was synthesized by the uncatalyzed reaction of 1heptadecanol (50 mg) with an excess of absolute formic acid (100  $\mu$ l). The reaction mixture was heated overnight at 80°C in a Reacti-Vial, after which the excess formic acid was removed in vacuo to give pure (GC) heptadecan-1-yl formate. <sup>13</sup>C:  $\delta$  (CDCl<sub>3</sub>) 161.18 (s, C-1'), 64.14 (t, C-1), 31.95 (t, C-15), 29.21-29.71 (11C, t, C-4-C-14), 28.54 (t, C-2), 25.84 (t, C-3), 22.71 (t, C-16), 14.12 (q, C-17).

Octadecan-4-olide was synthesized by the Mn(III) acetate-catalyzed free radical coupling of hexadec-1-ene and acetic acid (Heiba et al., 1983). KMnO<sub>4</sub> (1.09 g; 7 mmol) was added to a stirred solution of Mn(II) acetate (7.07 g; 29 mmol) in acetic acid (40 ml). After an initial increase in the temperature, the reaction mixture was allowed to cool to 90°C. The temperature increased on slow addition of acetic anhydride (10 ml) and the reaction mixture was again allowed to cool to 90°C, after which hexadec-1-ene (5.01 g; 23 mmol) was added to the mixture. This was immediately followed by the addition of anhydrous sodium acetate (16.7 g, 204 mmol). The reaction mixture was refluxed for 2 hr, diluted with a large volume of water (120 ml), the  $\gamma$ -lactone extracted with benzene, and the extract dried on anhydrous Na<sub>2</sub>SO<sub>4</sub>. Removal of the solvent and bulb-to-bulb distillation gave the pure (GC) octadecan-4-olide (4.95 g, 78%), bp 235°C/0.015 torr (air bath temperature). <sup>13</sup>C:  $\delta$  (CDCl<sub>3</sub>) 177.24 (s, C-1), 81.06 (d, C-4), 35.64 (t, C-2), 31.97 (t, C-16), 29.40–29.74 (10C, t, C-6–C-15), 28.04 (t, C-3), 25.27 (t, C-5), 22.72 (t, C-17), 14.12 (q, C-18).

Hexadecan-5-olide was synthesized by oxymercuration of tridec-1-ene, followed by free radical coupling of the product with acrylonitrile, and hydrolysis of the resulting 5-hydroxyhexadecane-1-nitrile according to the method of Giese et al. (1984). Mercury(II) acetate (4.35 g; 13.6 mmol) and tridec-1-ene (2.49 g; 13.6 mmol) were added to a solution of THF (35 ml) in water (15 ml), and the reaction mixture was stirred for 1 hr. Acrylonitrile (2.16 g; 40.7 mmol), sodium hydroxide solution (2 M, 15 ml), and sodium borohydride (1.03 g; 27.2 mmol) dissolved in sodium hydroxide solution (2 M, 55 ml) were added to the reaction mixture, which was stirred for a further 30 min. The organic material was extracted with dichloromethane. Removal of the solvent gave the crude hydroxynitrile, which was saponified by refluxing it with sodium hydroxide (2 M, 40 ml) for 4 hr. Unsaponified material was extracted with ether, after which the water layer was acidified with sulfuric acid (2 M) and the  $\delta$ -lactone extracted with ether, the ether extract dried on anhydrous  $Na_2SO_4$ , and the solvent removed in vacuo. Bulb-to-bulb distillation gave the required hexadecan-5-olide (2.36 g, 68%), bp 175°C/0.02 torr (air bath temperature). <sup>13</sup>C:  $\delta$  (CDCl<sub>3</sub>) 171.97(s, C-1), 80.61(d, C-5), 35.88(t, C-2), 31.94(t, C-14), 29.37–29.69(8C, t, C-6-C-13), 27.83(t, C-4), 24.96(t, C-6), 22.70(t, C-15), 18.53(t, C-3), 14.11(q, C-16).

## **RESULTS AND DISCUSSION**

A typical total ion chromatogram of an extract of the preorbital secretion is shown in Figure 1. Tentative identification of a number of the volatile organic constituents was based on a comparison of their mass spectra with those in NBS and Wiley mass spectra libraries. Further structural information on many of the volatile constituents of the secretion was furnished by their chemical ionization mass spectra obtained with methane and nitric oxide as reactant gases. Information on the position of the double bond(s) in some of the unsaturated constituents was obtained from their CI(NO) mass spectra (Malosse and Einhorn, 1990) and the EI mass spectra of their dimethyl disulfide derivatives (Buser et al., 1983; Carlson et al., 1989; Vincenti et al., 1987).

The most salient aspects of the identification of most of the compound types present in preorbital secretions based on their EI,  $CI(CH_4)$  and CI(NO) spectra have been discussed in considerable detail in the previous communication in this series (Mo et al., 1995) and will not be repeated here. However, since mass spectral information on long-chain compounds is not readily available, the relevant information for some of the constituents identified in the secretion, is given in Tables 1 to 6. The diagnostic ions in the EI mass spectra of the DMDS derivatives of the mono- and diunsaturated compounds that were used to determine the position of the double bonds in these compounds, are summarized in Tables 7 and 8.

In contrast to the preorbital secretion of the oribi, which contains compounds having a finite chain length (Mo et al., 1995), the preorbital secretion of the grysbok appears to contain large concentrations of compounds having chain lengths beyond those identified in the present investigation. These compounds have very low vapor pressures and, if the frequency of territorial marking in the grysbok is taken into account, it seems unlikely that these long-chain compounds could be essential ingredients of the territorial marking pheromone of the animal. They may, however, constitute a controlled-release medium for the more volatile constituents of the secretion. A consequence of the presence of these long-chain compounds in the secretion is that capillary columns coated with highly polar and thermally less stable stationary phases such as Carbowax,



BLE 1. DIAGNOSTIC IONS IN EI, CI(CH4), AND CI(NO) MASS SPECTRA OF 1-ALKANOLS PRESENT IN PREORBITAL SECRETION OF	Grysbok
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					Ion (%, r	normalized abus	ndance)			
No.		щ	IE		CI(CH <sub>4</sub> )			CI	NO)	
Figure 1	1-Alkanol	(W - 18) <sup>+</sup>	$(M - 46)^{+}$	Ť	(M - I) <sup>+</sup>	$(M - 17)^{+}$	$(M + 28)^{+}$	⁺ M	$(M - 1)^{+}$	(M - 3) <sup>+</sup>
-	I-Undecanol	154"	126 (46)	172 (4)	171 (76)	155 (100)	200 (100)	172 (5)	171 (20)	169 (90)
2	1-Dodecanol	168 (0.4)	140 (5)	186 (10)	185 (77)	169 (100)	214 (97)	186 (3)	185 (22)	183 (100)
П	I-Tridecanol	182 (0.4)	154 (4)	200 (11)	(08) 661	183 (100)	228 (100)	200 (3)	199 (22)	197 (92)
20	1-Tetradecanol	196 (0.4)	168 (4)	214 (14)	213 (92)	197 (100)	242 (100)	214 (4)	213 (30)	211 (75)
28	I-Pentadecanol	210 (0.4)	182 (3)	228 (16)	227 (100)	211 (98)	256 (100)	228 (4)	227 (30)	225 (76)
"Not deter	sted.									

BLE 2. DIAGNOSTIC IONS IN EI, CI(CH4), AND CI(NO) MASS SPECTRA OF 1-ALKANYL FORMATES PRESENT IN PREORBITAL SECRETION	OF GRYSBOK
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No. in Figure 1         EI         CI(CH <sub>4</sub> )           Rou in Figure 1         Formate $(M - 46)^+$ $(M - 74)^+$ $M^+$ $(M - 29)^+$				Diagno	ostic ion (%, r	normalized abu	ndance)		
Figure I         Formate $(M - 46)^+$ $(M - 74)^+$ $M^+$ $(M - 1)^+$ $(M - 29)^+$ $(M$ 3         Undecyl         154°         126 (12)         200°         199°         171°         1           8         Dodecyl         168 (2)         140 (8)         214 (1)         213 (5)         185°         1           17         Tridecyl         182 (2)         154 (6)         228 (1)         227 (6)         199 (2)         1           24         Tetradecyl         182 (2)         168 (5)         242 (1)         213 (4)         1           23         Pentadecyl         196 (1)         182 (3)         256 (1)         227 (3)         2	E	I	IE		CI	(CH4)		CI(	10)
3       Undecyl       154°       126 (12)       200°       199°       171°       1         8       Dodecyl       168 (2)       140 (8)       214 (1)       213 (5)       185°       10         17       Tridecyl       182 (2)       140 (8)       214 (1)       213 (5)       185°       10         24       Tetradecyl       196 (2)       154 (6)       228 (1)       227 (6)       199 (2)       11         23       Pentadecyl       196 (1)       242 (1)       241 (7)       213 (4)       1         33       Hexadecyl       210 (1)       182 (3)       256 (1)       277 (3)       273 (4)       1         34       Hexadecyl       210 (1)       182 (3)       255 (10)       227 (3)       2	1 Formate	(M - 46) <sup>+</sup>	$(M - 74)^{+}$	¥ M	$(M - 1)^{+}$	(M - 29) <sup>+</sup>	$(M - 45)^{+}$	$(M + 30)^{+}$	$(M - 1)^{+}$
8         Dodecyl         168 (2)         140 (8)         214 (1)         213 (5)         185"         1           17         Tridecyl         182 (2)         154 (6)         228 (1)         227 (6)         199 (2)         1           24         Tetradecyl         196 (2)         154 (6)         228 (1)         227 (6)         199 (2)         1           33         Pentadecyl         196 (2)         168 (5)         242 (1)         241 (7)         213 (4)         1           38         Hexadecyl         210 (1)         182 (3)         256 (1)         227 (3)         2	Undecyl	154°	126 (12)	200"	199"	171ª	155 (100)	230 (100)	199"
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	Dodecyl	168 (2)	140 (8)	214 (1)	213 (5)	185"	169 (100)	244 (100)	213"
24       Tetradecyl       196 (2)       168 (5)       242 (1)       241 (7)       213 (4)       1         33       Pentadecyl       210 (1)       182 (3)       256 (1)       255 (10)       227 (3)       2         38       Hexadecyl       210 (1)       182 (3)       256 (1)       255 (10)       227 (3)       2         49       Heptadecyl       238°       210 (2)       284 (3)       283 (15)       255 (8)       2         56       Octadecyl       252°       224 (2)       298 (3)       297 (20)       269 (8)       2         56       Nonadecyl       252°       238 (2)       311 (4)       311 (23)       283 (6)       2         72       lcosyl       280°       238 (2)       312 (4)       312 (4)       336 (5)       2         72       lcosyl       252°       238 (2)       312 (4)       337 (20)       266 (8)       2         72       lcosyl       280°       238 (2)       312 (4)       333 (6)       2         80       Henicosyl       286°       133 26 (1)       325 (10)       257 (10)       29         81       Tricosyl       290°       252 (1)       326 (1)       325 (1)       325 (	Tridecyl	182 (2)	154 (6)	228 (1)	227 (6)	199 (2)	183 (100)	258 (100)	227 (1)
33       Pentadecyl       210 (1)       182 (3)       256 (1)       255 (10)       227 (3)       2         38       Hexadecyl       224"       196 (1)       270 (2)       269 (13)       241 (6)       2         49       Heptadecyl       238"       210 (2)       284 (3)       283 (15)       255 (8)       2         56       Octadecyl       252"       224 (2)       298 (3)       297 (20)       269 (8)       2         66       Nonadecyl       252"       238 (2)       311 (4)       311 (23)       283 (6)       2         72       lcosyl       280"       252 (1)       326 (4)       325 (20)       297 (10)       29         80       Henicosyl       280"       252 (1)       340 (7)       339 (28)       311 (10)       29         81       Docosyl       308"       280"       354 (10)       253 (30)       325 (11)       39         90       Tricosyl       323"       368"       382 (15)       381 (6)       339 (17)       3         90       Tetracosyl       326"       368"       382 (15)       351 (23)       339 (17)       3	Tetradecyl	196 (2)	168 (5)	242 (1)	241 (7)	213 (4)	197 (100)	272 (100)	241 (6)
38         Hexadecyl         224"         196 (1)         270 (2)         269 (13)         241 (6)         2           49         Heptadecyl         238"         210 (2)         284 (3)         283 (15)         255 (8)         2           56         Octadecyl         252"         210 (2)         284 (3)         283 (15)         255 (8)         2           66         Nonadcyl         252"         224 (2)         298 (3)         297 (20)         269 (8)         2           72         lcosyl         252"         238 (2)         312 (4)         311 (23)         283 (6)         2           80         Henicosyl         280"         252 (1)         326 (4)         325 (20)         297 (10)         2           81         Docosyl         294"         266 (1)         340 (7)         339 (28)         311 (10)         2           83         Docosyl         328"         233 (10)         355 (11)         3           90         Tricosyl         326"         338" (10)         367 (32)         339 (17)         3           90         Tetracosyl         336"         308"         382 (15)         381 (6)         333 (17)         3	Pentadecyl	210 (1)	182 (3)	256 (1)	255 (10)	227 (3)	211 (100)	286 (100)	255 (3)
49         Heptadecyl         238°         210 (2)         284 (3)         283 (15)         255 (8)         2           56         Octadecyl         252"         224 (2)         298 (3)         297 (20)         269 (8)         2           66         Nonadecyl         252"         238 (2)         312 (4)         311 (23)         283 (6)         2           72         lcosyl         280"         252 (1)         326 (4)         325 (20)         297 (10)         2           80         Henicosyl         294"         266 (1)         340 (7)         339 (28)         311 (10)         2           83         Docosyl         308"         280"         354 (10)         253 (30)         325 (11)         3           87         Tricosyl         323"         308"         308"         381 (10)         253 (30)         325 (11)         3           90         Tetracosyl         336"         308"         381 (6)         353 (22)         3         339 (17)         3           91         Tetracosyl         336"         381 (6)         353 (22)         3         3         3         3         3         3         3         3         3         3         3	Hexadecyl	224ª	196 (1)	270 (2)	269 (13)	241 (6)	225 (100)	300 (100)	269"
56         Octadecyl         252"         224 (2)         298 (3)         297 (20)         269 (8)         2           66         Nonadecyl         266"         238 (2)         312 (4)         311 (23)         283 (6)         2           72         Icosyl         280"         252 (1)         326 (4)         325 (20)         297 (10)         2           80         Henicosyl         294"         266 (1)         340 (7)         339 (28)         311 (10)         2           83         Docosyl         308"         280"         354 (10)         253 (30)         325 (11)         3           87         Tricosyl         322"         294"         368 (10)         367 (32)         339 (17)         3           90         Tetracosyl         336"         382 (15)         381 (6)         353 (22)         3	Heptadecyl	238"	210 (2)	284 (3)	283 (15)	255 (8)	239 (100)	314 (100)	283 (6)
66         Nonadecyl         266"         238         (2)         312         (4)         311         (23)         283         (6)         21           72         Icosyl         280"         252         (1)         326         (4)         325         (20)         297         (10)         21           80         Henicosyl         294"         256         (1)         340         (7)         339         28)         311         (10)         21           83         Docosyl         308"         280"         354         (10)         253         30)         325         (11)         31           87         Tricosyl         322"         294"         368         (10)         367         (32)         339         (17)         33           90         Tetracosyl         336"         308"         382         (15)         381         (0)         353         (22)         33         (17)         33	Octadecyl	252"	224 (2)	298 (3)	297 (20)	269 (8)	253 (100)	328 (100)	297 (2)
72         Icosyl         280"         252 (1)         326 (4)         325 (20)         297 (10)         21           80         Henicosyl         294"         266 (1)         340 (7)         339 (28)         311 (10)         21           83         Docosyl         308"         280"         354 (10)         253 (30)         325 (11)         31           87         Tricosyl         322"         294"         368 (10)         367 (32)         339 (17)         33           90         Tetracosyl         336"         300"         332 (15)         33         339 (17)         3           90         Tetracosyl         336"         300"         357 (20)         353 (22)         3	Nonadecyl	266"	238 (2)	312 (4)	311 (23)	283 (6)	267 (100)	342 (100)	311 (2)
80         Henicosyl         294"         266 (1)         340 (7)         339 (28)         311 (10)         22           83         Docosyl         308"         280"         354 (10)         253 (30)         325 (11)         31           87         Tricosyl         322"         294"         368 (10)         367 (32)         339 (17)         33           90         Tetracosyl         336"         308"         382 (15)         381 (60)         353 (22)         3           90         Doccostul         360"         300"         300"         353 (22)         3	Icosyl	280"	252 (1)	326 (4)	325 (20)	297 (10)	281 (100)	356 (100)	325 (12)
83         Docosyl         308°         280°         354 (10)         253 (30)         325 (11)         31           87         Tricosyl         322°         294°         368 (10)         367 (32)         339 (17)         33           90         Tetracosyl         336°         308°         382 (15)         381 (60)         353 (22)         3           90         Democratul         360°         37.00         300.00         353 (22)         3	Henicosyl	294"	266 (1)	340 (7)	339 (28)	311 (10)	295 (100)	370 (100)	339 (15)
87         Tricosyl         322"         294"         368 (10)         367 (32)         339 (17)         33           90         Tetracosyl         336"         308"         382 (15)         381 (60)         353 (22)         33           90         Demonstruct         260"         374"         300"         381 (60)         353 (22)         33	Docosyl	308"	280°	354 (10)	253 (30)	325 (11)	309 (100)	384 (100)	353 (4)
90 Tetracosyl 336° 308° 382 (15) 381 (60) 353 (22) 3.	Tricosyl	322"	294"	368 (10)	367 (32)	339 (17)	323 (100)		
07 Data 200 2508 2228 200 200 200 200 200 200 200 200	Tetracosyl	336°	308"	382 (15)	381 (60)	353 (22)	337 (100)		
F (C1) / 05 (CC) C65 (7) 065 .75 005 16000000 76	Pentacosyl	350"	322"	396 (7)	395 (55)	367 (15)	351 (100)		

## MAMMALIAN EXOCRINE SECRETIONS X

"Not detected.

			EI				CI(CH	0	
No. in Figure 1	Alkenol	м⁺	$(M - 18)^+$	(M - 46) <sup>+</sup>	(M + 1) <sup>+</sup>	M⁺	(M - 1)*	(M - 17)*	(M ~ 18)*
4	6-Dodecen-1-ol	184°	166"	138 (10)	185 (7)	184 (2)	183 (9)	167ª	166 (15)
10"	6-Tridecen-1-ol 7-Tridecen-1-ol	198"	180 (3)	152 (4)	199 (10)	198 (2)	197 (13)	181 (4)	180 (19)
19*	8-Tetradecen-1-ol 7-Tetradecen-1-ol	212"	194 (2)	166 (1)	213 (12)	212 (2)	211 (13)	195 (5)	194 (17)
26 <sup>b</sup>	8-Pentadecen-1-ol	226ª	208 (2)	180 (1)	227 (16)	226 (3)	225 (15)	209 (4)	208 (21)
41	8-Heptadecen-1-ol	254"	236 (2)	208 (1)	255 (16)	254 (3)	253 (19)	237 (4)	236 (25)
58	10-Nonadecen-1-ol	282°	264"	236"	283 (19)	282 (3)	281 (20)	265 (7)	264 (28)

TABLE 3. DIAGNOSTIC IONS IN EI, CI(CH<sub>4</sub>), AND CI(NO) MASS

"Not detected.

<sup>b</sup>Coeluting isomers are given in the order in which their DMDS derivatives are eluted.

could not be employed in GC and GC-MS analyses, as these phases cannot survive repeated high-temperature conditioning. Limited use was made of capillary columns coated with OV-1701-OH, but the apolar PS-089-OH column produced the best results, although some of the isomeric alkenols were found to coelute from this column. Fortunately the DMDS derivatives of these compounds were separated on the apolar column and could therefore be unequivocally identified.

Probably because it has a relatively large ion volume, the CI ion source of the QMD 1000 mass spectrometer gives poor CI spectra with a build-up of the baseline towards the end of the total ion chromatogram. It was nevertheless possible to obtain useful information from the  $CI(CH_4)$  spectra of some of the more volatile constituents of the secretion. The use of NO as CI reactant gas deserves mention. The CI(NO) mass spectra furnish information from which the molecular mass of a compound, in many cases the compound type, and the position of the double bond in monounsaturated compounds can be derived (Hunt et al., 1982). Due to the previously noted problems with the CI source, ions furnishing information on the position of the double bond in unsaturated compounds could be detected only in the spectra of compounds that were present in relatively high concentrations and/or were well separated from the other constituents of the secretion. The results obtained in these analyses nevertheless illustrated the potential of NO as CI reactant gas.

					CI	NO)		
(M - 19)*	(M + 28)*	м⁺	(M - I)*	(M - 2)*	(M - 3)*	(M - 19)*	[CH <sub>3</sub> (CH <sub>2</sub> ),CO] <sup>+</sup>	$[CH_3(CH_2), CH = N-OH]$
165 (12)	212"	184"	183"	182"	181"	165"		
179 (15)	226"	198"	197 (57)	196 (2)	195 (6)	179"	99 (17)	158 (23)
193 (12)	240 (12)	212 (14)	211 (100)	210 (40)	209 (15)	193"		158 (37)
207 (13)	254 (29)	226 (17)	225 (100)	224 (37)	223 (40)	207 (41)	141 (10)	158 (24)
235 (13)	282 (95)	254 (20)	253 (100)	252"	251"	235"		
263 (14)	310 (7)	282 (1)	281 (22)	280 (15)	279 (3)	263 (28)		

SPECTRA OF ALKENOLS PRESENT IN PREORBITAL SECRETION OF GRYSBOK

Diagnostic ion (%, normalized abundance)

Only two saturated and one unsaturated aldehyde are present in low concentrations in the preorbital secretion of the grysbok, whereas the oribi's secretion contains several saturated, monounsaturated, and diunsaturated aldehydes. The possibility was considered that this difference could be the result of autoxidation of the aldehydes taking place much more readily in the open preorbital cavity of the grysbok than in the deeper preorbital structure of the oribi. However, since neither the secretion of the oribi nor that of the grysbok contains detectable amounts of carboxylic acids and aldehydes having the same chain lengths, this does not seem to be a tenable hypothesis.

The EI mass spectra of components 43, 52, 61, 69, and 81 all have a base peak of high relative abundance at m/z 85. These compounds were identified as pentadecan-4-olide, hexadecan-4-olide, heptadecan-4-olide, octadecan-4-olide, and icosan-4-olide, respectively using, among others, data obtained from their CI mass spectra.

In addition to these compounds having a base peak at m/z 85 in their EI mass spectra, the preorbital secretion contains component 54 with an equally abundant base peak at m/z 99 in its EI mass spectrum. Taking the unbranched structures of the other constituents of the preorbital secretion into consideration, the possibility that this component could be an alkan-5-olide or  $\delta$ -lactone, was considered. The molecular ion is not observed in its EI mass spectrum, but the CI(CH<sub>4</sub>) mass spectrum affords conclusive evidence that the compound has a

<ol> <li><sup>5</sup><sup>4</sup> <sup>1</sup> <sup>1</sup> <sup>1</sup> <sup>1</sup> <sup>1</sup> <sup>1</sup> <sup>1</sup> <sup>1</sup> <sup>1</sup> <sup>1</sup></li></ol>	45 e-rice 45 8-Hep 53 <sup>6</sup> (9-Octa 63 (0-Noi 63 (0-Noi 70 <sup>6</sup> - (11-kos 78 (12-Her 78 (12-Her	tadecenyl udecenyl nadecenyl senyl senyl senyl nictssenyl	268" 282" 346" 348"	222" 236 (6) 264" 264"	241 (1) 241 (1) 255 (3) 268 (3) 269 (3) 269 (3) 283 (3) 283 (3) 299 (3)	225 (1) 239 (1) 239 (1) 253 (1) 267 (1) 267 (2) 281 (2) 309 (2) 323 (3)	(M - 29); 197 (1) 211 (1) 211 (1) 211 (1) 211 (1) 211 (1) 211 (1) 211 (1) 2135 238 (1) 238 (1) 238 (1) 238 (1) 238 (1)	(M - 45) <sup>+</sup> (M - 45) <sup>+</sup> (H (7) (95 (7) 209 (9) 209 (9) 209 (10) 233 (10) 265 (12) 265 (12) 279 (7) 293 (12)	(M 46) (M 46) (M 46) (M 24) (M (24) 208 (23) 208 (23) 208 (23) 208 (24) 208 (25) 208 (24) 208 (25) 208 (24) 208 (25) 208 (26) 208 (27) 208 (27) 208 (28) 208 (	(M + 30) <sup>-1</sup> 270 (55) 284 (27) 340 (87) 340 (67)	M <sup>-</sup> 240 <sup>-</sup> 254 (6) 282 (0) 300 (23) 308 (15)	(1) - (M) (001) W2 (40) R2 (001) IN2 (001) OI6 (001) OI6	Ctt (M = 46) <sup>-</sup> 194 (13) 208 (15) 208 (15) 264 (21) 264 (21)	NO) A' = (CH-L-CO)' 	B = [00 <sup>-1</sup> (CH <sub>1</sub> ) <sub>10</sub> (00CH] (71 (13) (99 (13) 227 (15)
82 13-Ducuscryd 353 (10) 351 (3) 323 (5) 307 (15) 306 (4) 86 14-Trenssenyd 306° 320 (3) 367 (10) 365 (3) 317 (4) 321 (12) 320 (60) 89° 1155-Ternacoscryf 300° 334 (4) 381 (3) 379 (3) 351 (6) 335 (20) 334 (3) 91° 1165-Pernacoscryf 304° 348 (3) 395 (11) 393 (5) 365 (6) 349 (16) 348 (65)	82' I.J.Du 86 I. <del>I.T</del> riv 89' (15)-Tr 91'' (16)-P.	cosenyl cosenyl etracosenyl entacosenyl	366" 380" 394"	320 (3) 334 (4) 348 (3)	353 (10) 367 (10) 381 (3) 395 (11)	351 (3) 365 (3) 379 (3) 393 (3)	323 (5) 337 (4) 351 (6) 365 (6)	307 (15) 321 (12) 335 (20) 349 (16)	306 (48) 320 (60) 334 (38) 348 (65)						

TABLE 4. DIAGNOSTIC IONS IN EI, CI(CH<sub>4</sub>), AND CI(NO) MASS SPECTRA OF ALKENYL FORMATES PRESENT IN PREORBITAL SECRETION OF GRYSBOK

"Not detected. "Coeluting names are given in the order in which their DMDS derivatives are eluted.

' Not observed in the EI total ion gas chromatogram. "Not observed in EI total ion chromatogram of DMDS derivatives; double bond position uncertain.

molecular mass of 254. The pair of peaks at m/z 70 and 71, possibly formed by the loss of, respectively, CHO and CO from the ion at m/z 99, are typical for  $\delta$ -lactones of this type. Final confirmation that this component is hexadecan-5-olide was furnished by retention-time comparison with an authentic synthetic sample of the compound.

The preorbital secretion of the grysbok contains two constituents, 57 and 73, each having ions at m/z 48, 61, 75, and 103 in the lower mass range and two ions of high abundance differing by 15 mass units in the high mass range of their EI mass spectra. Information afforded by the CI(CH<sub>4</sub>) mass spectra of the two compounds indicates that the ions at m/z 286 and 314 in their EI mass spectra are their respective molecular ions. Properties such as the molecular mass of the compounds, the facile loss of a methyl radical, the stability of the molecular ions, etc., suggest these compounds to be alkyl methyl sulfides. Taking component 57 as an example, the formation of some of the sulfur-containing ions in its spectrum can be formulated as shown in Scheme 1.

Components 57 and 73 were identified as heptadecyl methyl and methyl nonadecyl sulfides, respectively. It is interesting that reasonably abundant  $M^+$ ,  $(M-H)^+$ ,  $(M-2H)^+$ ,  $(M-3H)^+$ , and  $(M-SCH_3)^+$  ions are present in the CI(CH<sub>4</sub>) spectra of both compounds. The  $(M-SCH_3)^+$  ions, present in these mass spectra, are not observed in their EI spectra, possibly because the resulting primary carbocations undergo rapid fragmentation under EI conditions.

In certain respects the EI mass spectra of components 75 and 84 are similar to those of several hydroxyesters present in the dorsal secretion of the springbok, *Antidorcas marsupialis* (Le Roux, 1980; Burger et al., 1981b), and the interdigital secretion of the bontebok, *Damaliscus dorcas dorcas* (Nell, 1992). The hypothesis that these two components could possibly be similar hydroxyesters is supported by their EI and CI(CH<sub>4</sub>) mass spectra. The high-resolution EI spectra of the hydroxyesters found in the dorsal secretion of some of the diagnostically significant ions in the mass spectrum of, for example, 2-hydroxy-icosan-1-yl acetate 84 (Figure 2) as shown in Scheme 2.

It is clear that the hydroxy group cannot be present in either the carboxyl function of the molecule or on the  $\alpha$ -carbon atom of the alcohol residue of the molecule. Its presence on the  $\beta$ -carbon atom is confirmed by the ion at m/z 103. The formation of the prominent ion at m/z 74 in its mass spectrum can be rationalized in terms of the expulsion of a molecule of formaldehyde, followed by a McLafferty rearrangement (Scheme 3).

It can be accepted that the ions at m/z 357 and 355 in the CI(CH<sub>4</sub>) mass spectrum of this compound (Figure 3) are generated by M+H and M-H processes, respectively, and that the ions at m/z 297 and 295 are formed by the expulsion of acetic acid from these respective ions. The unbranched structure

		. <u></u>	EI			Cl(CH₄)	)
No. in Figure 1	Alkadienol	M+	(M - 18) <sup>+</sup>	$(M + 1)^{+}$	M⁺	$(M - 1)^+$	(M - 17) <sup>+</sup>
9	4,7-Tridecadienol	196"	178 (0.5)	197 (25)	196 (4)	195 (13)	179 (23)
18	5,8-Tetradecadienol	210	192 (0.5)	211 (20)	210 (10)	209 (15)	193 (25)
25	6,9-Pentadecadienol	224 (0.4)	206 (0.5)	225 (30)	224 (17)	223 (23)	207 (30)
40	8,10-Heptadecadienol	252 (0.4)	234 (0.2)	253 (31)	252 (28)	251 (56)	235 (31)

TABLE 5. DIAGNOSTIC IONS IN EI, CI(CH4), AND CI(NO) MASS SPECTRA

"Not detected.

of the alcohol residue of the molecule was confirmed by coinjection of an extract of the secretion with a synthetic sample of 2-hydroxyicosan-1-yl acetate synthesized in an  $Al_2O_3$ -catalyzed reaction of 1,2-epoxyicosane and acetic acid (Greyling, 1992). Component 75 was identified along similar lines as 2-hydroxyoctadecan-1-yl acetate.

Six  $\gamma$ -lactones, a  $\delta$ -lactone, and the two hydroxyesters are the only chiral compounds identified in the preorbital secretion of the grysbok. The structure of the preorbital cavity is not so favorable for the survival of bacteria as that of the interdigital cavity of the bontebok and blesbok, but several bacteria, e.g., *Escherichia coli*, have been identified in the preorbital secretion of the grysbok (Ruppel, 1984). Lactones were found to be produced by the bacteria in the interdigital cavity in the bontebok and blesbok (Nell, 1992), and it is therefore possible that similar processes could be responsible for the presence of some of the constituents of the preorbital secretion of the grysbok. Regardless of whether these chiral constituents are synthesized in the gland or by bacteria, they are expected to be optically active. The determination of the absolute configuration of these compounds was not undertaken in the present study, however.

The double bonds in all of the unsaturated constituents of the secretion were assumed to possess Z configuration. In a previous study (Le Roux, 1980) representative examples of the various unsaturated compound types were isolated in quantities large enough to allow the recording of their <sup>13</sup>C NMR spectra. The presence in these spectra of a resonance at ca. 27.2 ppm, which can be ascribed to the allylic carbon atoms, was taken as unambiguous evidence that the double bonds in these compounds have the Z configuration, as the resonance of allylic carbon atoms in compounds with *E* configuration appears at ca. 32.5 ppm (Breit-

Diagnostic ion (%, normalized abundance)

				CI(NO)			
$(M - 19)^{+}$	(M + 30) <sup>+</sup>	(M + 28) <sup>+</sup>	M+	(M - 1) <sup>+</sup>	(M - 2) <sup>+</sup>	(M - 18) <sup>+</sup>	(M - 19) <sup>+</sup>
177 (31)	226ª	224"	196 (23)	195 (91)	194"	178 (65)	177"
191 (30)	240 (45)	238"	210 (80)	209 (100)	208 (52)	192 (25)	191 (12)
205 (18)	254 (19)	252 (13)	224 (50)	223 (100)	222 (33)	206 (7)	205 (23)
233 (23)	282 (35)	280 (10)	252 (75)	251 (84)	250 (6)	234"	233"

OF ALKADIENOLS PRESENT IN PREORBITAL SECRETION OF GRYSBOK

maier et al., 1979; Wenkert et al., 1976). Careful comparison of retention times and of retention time increments confirmed this assumption. In this regard it is noteworthy that in the chemical characterization of the preorbital secretion of the oribi, *Ourebia ourebi*, in which more reference compounds were available, all the unsaturated constituents were found to possess the Z configuration (Mo et al., 1995). Both the oribi and the grysbok belong to the tribe Neotragini.

The compounds identified in the preorbital secretion of the grysbok are listed in Table 9, together with compounds identified in the previous study (Le Roux, 1980; Burger et al., 1981a) and information on the analytical methods employed.

Unfortunately, a number of compounds still remained unidentified, mainly due to the lack of structurally significant information in their mass spectra and to the poor quality of the CI total ion current chromatogram. As the whole secretion was subjected to DMDS derivatization for the determination of the position of double bonds in the unsaturated constituents, the DMDS derivatives of the minor constituents could in some cases not be found in the total ion current chromatogram of the derivatized material, probably because the derivatives coeluted with certain constituents of the secretion. The unidentified constituents are also listed in Table 9 with mass spectral data, including the molecular ion or the ion at highest mass in a mass spectrum. The minor constituents, formic acid, hexanal, 2-heptanone, 2-octanone, 2-nonanone, and nonanoic acid, identified in the previous study of the preorbital secretion of the grysbok, are not visible in the total ion chromatogram depicted in Figure 1 and are not included in Table 9. In the previous study the coeluting alcohols (Z)-6-tridecen-1-ol.

AND CI(NO) MASS SPECTRA OF ALKADIENYL FORMATES PRESENT IN PREORBITAL	SECRETION OF GRYSBOK
CI(CH4),	
. DIAGNOSTIC IONS IN EI,	
<b>TABLE 6</b>	

			Е		Ū	C			CIC	ô	
No. in Figure 1	Formate	Ť	(M - 46) <sup>+</sup>	(I + W)	Ť	+(1 – W)	(M - 45) <sup>+</sup>	(M + 30) <sup>+</sup>	+(i + W)	Ť	(W - I) <sup>+</sup>
21	(5,8)-Tetradecadienyl <sup>6</sup>	238 <sup>6</sup>	192 <sup>6</sup>	239*	238 (9)	237 (38)	193 (47)	268 (22)	239*	238 (100)	237"
29	6.9-Pentadecadienyl	252 (3)	206 (0.5)	253 (15)	252 (25)	251 (30)	207 (60)	282 (48)	253 (20)	252 (100)	251 (40)
4	8,11-Heptadecadienyl	280 (3)	234 <sup>b</sup>	281 (7)	280 (48)	279 (35)	235 (35)	310 (60)	281 (20)	280 (100)	279 (42)
62	(10,13)-Nonadecadienyl4	308 (3)	262 <sup>*</sup>	309 (20)	308 (25)	307 (17)	263 (23)	338 (33)	309 (60)	308 (100)	307 (17)
77	(12,15)-Henicosadienyl <sup>a</sup>	336 (3)	290 <sup>¢</sup>	337°	336 (1)	335 (6)	291 (4)	366 (41)	337 (11)	336 (56)	335°
"Not loc	ated in the EI total ion cl	hromatogra	um of the DN	IDS derivati	ves; double	e bond posit	ion uncertain				
"Not det	ected.										

BURGER, TIEN, LE ROUX, AND MO

	$ \begin{array}{c} \text{SCH}_3 \\ \text{CH}_3 & \longrightarrow \\ \text{CH}_2 & \longrightarrow$	$CH_3$ $CH - CH_2$ $H^+$	m - R = OH R = OC	(alcohols) HO (formates)	
No. in		Dia	ngnostic ion (%, n	ormalized abunda	nce)
Figure 1	Alkenol/alkenyl formate	M*	$(M - 47)^+$	A+	B*
4	6-Dodecen-1-ol	278 (70)	231 <sup>a</sup>	131 (100)	147 (47)
100	6-Tridecen-1-ol	292 (57)	245 <sup>a</sup>	145 (100)	147 (46)
10	7-Tridecen-1-ol	292 (70)	245 <sup>a</sup>	131 (66)	161 (67)
100	§ 8-Tetradecen-1-ol	306 (77)	259 (3)	145 (100)	161 (66)
19	7-Tetradecen-1-ol	306 (49)	259 (7)	131 (99)	175 (100)
260	§ 8-Pentadecen-1-ol	320 (48)	273 (8)	145 (100)	175 (95)
20	6-Pentadecen-1-ol	320 (28)	273 (3)	173 (100)	147 (45)
2 44	(8-Hexadecen-1-ol	334 (44)	287 (9)	159 (100)	175 (70)
34	7-Hexadecen-I-ol	334 (60)	287 (15)	159 (100)	175 (70)
41	8-Heptadecen-1-ol	348 (23)	301 (5)	173 (100)	175 (72)
58	10-Nonadecen-1-ol	376 (20)	329 (6)	173 (100)	203 (97)
71	12-Henicosen-1-ol	404 (19)	357 (5)	173 (85)	231 (100)
88	14-Tricosen-1-ol	432 (19)	385"	173 (65)	259 (100)
16	7-Tridecenyl formate	320 (24)	273"	131 (100)	189 (45)

334 (33)

334 (30)

348 (13)

348 (33)

362 (40)

362 (29)

376 (17)

390 (23)

390 (30)

404 (12)

418 (17)

418 (32)

432 (7)

446 (13)

460 (6)

287<sup>4</sup>

287 (7)

301 (3)

301 (8)

315 (17)

315 (10)

329 (5)

343 (5)

343 (8)

257 (3)

371 (10)

317 (22)

385 (2)

399 (3)

413 (3)

145 (100)

131 (85)

173 (85)

145 (100)

173 (100)

159 (100)

173 (100)

173 (79)

159 (33)

173 (60)

173 (38)

187 (68)

173 (24)

173 (35)

173 (39)

189 (65)

203 (100)

175 (14)

203 (95)

189 (40)

203 (98)

203 (90)

217 (100)

231 (65)

231 (100)

245 (100)

231 (100)

259 (100)

273 (100)

287 (100)

# TABLE 7. DIAGNOSTIC IONS IN EI MASS SPECTRA OF DMDS DERIVATIVES OF MONOUNSATURATED ALCOHOLS AND FORMATES PRESENT IN PREORBITAL SECRETION OF GRYSBOK

"Not detected.

22

23

30

31

36

45

53

63

70<sup>c</sup>

78

82<sup>c</sup>

86

<sup>b</sup>Coeluting isomers given in the order in which their DMDS derivatives are eluted.

Not located in EI and CI total ion chromatograms.

7-Tetradecenyl formate

8-Tetradecenyl formate

6-Pentadecenyl formate

8-Pentadecenvl formate

7-Hexadecenyl formate

8-Hexadecenyl formate

8-Heptadecenyl formate

9-Octadecenyl formate

10-Octadecenvl formate

10-Icosenyl formate

[11-Icosenyl formate

10-Nonadecenyl formate

12-Henicosenyl formate

13-Docosenyl formate

14-Tricosenyl formate

		Σ.	REORBITAL SE	CRETION OF U	RYSBOK				
	CH <sub>3</sub> S CH <sub>3</sub> (CH <sub>2</sub> ),		AB <sup>+</sup> BD <sup>+</sup> -	SCH (CH <sub>2</sub>	3 R	R = OH (alco R = OCOH (f	hols) ormates)		
No in	Alkadianul			Diagnostic	c ion (%, noi	malized abu	ndance)		
Figure 1	formate	, ₩	(M - 47) <sup>+</sup>	(M - 95) <sup>+</sup>	BD⁺	D⁺	AB+	A *	(BD - 48) <sup>+</sup>
6	4,7-Tridecadien-1-ol	322 (70)	275 <sup>4</sup>	227"	203 (32)	131 (47)	191 (32)	,611	155 (42)
25	6,9-Pentadecadien-1-ol	350 (90)	303 (35)	255 (32)	203 (31)	131 (62)	219 (4)	147 (19)	155 (100)
40	8,11-Heptadecadien-1-ol	378 (80)	3314	283 (43)	203 (25)	131 (64)	247 <sup>a</sup>	175 (4)	155 (57)
29	6,9-Pentadecadienyl formate	378 (65)	331 (35)	283 (32)	203 (31)	131 (79)	247 (4)	175 (7)	155 (100)
4	8,11-Heptadecadienyl formate	406 (18)	359 (27)	311 (27)	203 (26)	131 (22)	275"	203 (61)	155 (100)

TABLE 8. DIAGNOSTIC IONS IN EI MASS SPECTRA OF DMDS DERIVATIVES OF DIUNSATURATED ALCOHOLS AND FORMATES PRESENT IN oe Geveov NOLL ć ć

"Not detected.





SCHEME 2.







No. in Figure 1	Compounds	Quantity (pg/animal)	Analytical methods
1	Undecan-1-ol	30	a,b,c,d,i
5	Dodecan-1-ol	400	a,b,c,d,f,g,i
11	Tridecan-1-ol	1310	a,b,c,d,f,g,i
20	Tetradecan-1-ol	775	a,b,c,d,f,g,i
28	Pentadecan-1-ol	570	a,b,c,d,f,g,i
3	Undec-1-yl formate	7	b,c,i
8	Dodec-1-yl formate	40	b,c,d,i
17	Tridec-1-yl formate	140	b,c,d,i
24	Tetradec-1-yl formate	150	a,b,c,d,f,g,i
33	Pentadec-1-yl formate	370	a,b,c,d,f,i
38	Hexadec-1-yl formate	20	a,b,c,d,f,g
49	Heptadec-1-yl formate	75	b,c,d,i
56	Octadec-1-yl formate	95	a,b,c,d,f
66	Nonadec-1-yl formate	740	a,b,c,d,f,g
72	Icos-1-vl formate	340	a.b.c.d.f.g.i
80	Henicos-1-vl formate	1220	a.b.c.d.f.g.h.i
83	Docos-I-vl formate	565	a.b.c.f.g.i
87	Tricos-I-vI formate	4110	a.b.c.f.g.h
90	Tetracos-1-vl formate	1555	a b c f i
92	Pentacos-1-yl formate	6108	a.b.c.f
4	(Z)-6-Dodecen-1-ol	10	b.c.e
	(Z)-6-Tridecen-1-ol		b.c.d.e
10	(Z)-7-Tridecen-1-ol	270	b.c.d.e
	(Z)-8-Tetradecen-1-ol		b.c.d.e
19	(Z)-7-Tetradecen-1-ol	445	b.e
24	(Z)-8-Pentadecen-1-ol	1970	b,c,d,e
26	(Z)-6-Pentadecen-1-ol	1260	a,b,c,e,f,g,h
	(Z)-8-Hexadecen-1-ol		b.e
34	(Z)-7-Hexadecen-1-ol	50	b.e
41	(Z)-8-Heptadecen-1-ol	435	a,c,e,f,g,h
58	(Z)-10-Nonadecen-1-ol	50	a.c.f
71	(Z)-12-Henicosen-1-ol	25	b.e
88	(Z)-14-Tricosen-1-ol	30	b.e
16	(Z)-7-Tridecen-1-yl formate	50	b,e
22	(Z)-7-Tetradecen-1-vl formate	15	b.c.e
23	(Z)-8-Tetradecen-1-yl formate	20	b.c.e
30	(Z)-6-Pentadecen-1-yl formate	195	b.c.d.e
31	(Z)-8-Pentadecen-1-yl formate	50	b.c.e
	(Z)-7-Hexadecen-1-yl formate		b.c.e
36	(Z)-8-Hexadecen-1-yl formate	10	b.e
45	(Z)-8-Heptadecen-I-yl formate	715	a.b.c.d.e.f.g.b
62	(Z)-9-Octadecen-1-yl formate		b.c.e
55	(Z)-10-Octadecen-1-yl formate	10	b,e
63	(Z)-10-Nonadecen-1-yl formate	780	a,b,c,d,e,f,g
70	(Z)-10-Icosen-1-yl formate	10	b,e
/0	(Z)-11-Icosen-1-yl formate	10	b,e

TABLE 9. CONSTITUENTS OF PREORBITAL SECRETION OF GRYSBOK

No. in Figure 1	Compounds	Quantity (pg/animal)	Analytical methods
78	(Z)-12-Henicosen-1-yl formate	665	a,b,c,d,e,f,g
82	(Z)-13-Docosen-1-yl formate	20	b,e
86	(Z)-14-Tricosen-1-yl formate	1505	a,b,c,e,f,g
89	(Z)-15-Tetracosen-1-yl formate	25	b,c,k
91	(Z)-16-Pentacosen-1-yl formate	1190	b,c,k
9	(4Z,7Z)-4,7-Tridecadien-1-ol	60	b,c,d,k
18	(5Z,8Z)-5,8-Tetradecadien-1-ol	140	b,c,d,k
25	(6Z,9Z)-6,9-Pentadecadien-1-ol	2645	a,b,c,d,e,f,g,h
40	(8Z, 10Z)-8, 10-Heptadecadien-1-ol	265	a,b,c,d,e,f,g,h
21	(5Z,8Z)-5,8-Tetradecadien-1-yl formate	10	b,c,d,j,k
29	(6Z,9Z)-6,9-Pentadecadien-1-yl formate	180	b,c,d,e
44	(8Z,11Z)-8,11-Heptadecadien-1-yl formate	150	b,c,d,e
62	(10Z,13Z)-10,13-Nonadecadien-1-yl formate	80	b,c,d,k
77	(12Z,15Z)-12,15-Henicosadien-1-yl formate	70	b,c,d,k
2	Dodecanal	15	b,c,d,i
7	Tridecanal	50	b,c,d
6	(Z)-3-Tridecenal	35	b,c,k
27	Tetradecanoic acid	trace	b,d,i
35	Pentadecanoic acid	trace	b,d,i
42	Hexadecanoic acid	655	a,b,c,d,f,i
50	Heptadecanoic acid	15	b,d
59	Octadecanoic acid	110	a,b,d,f,i
74	Icosanoic acid	60	b,d
43	Pentadecan-4-olide	50	ь
52	Hexadecan-4-olide	120	b,c,d,i
61	Heptadecan-4-olide	20	b,c,d
69	Octadecan-4-olide	5	b,c,i
81	Icosan-4-olide	25	b,i
54	Hexadecan-5-olide	20	b,c,d,i
57	Heptadec-1-yl methyl sulfide	80	b,c,i
73	Methyl nonadec-1-yl sulfide	30	b,c
75	2-Hydroxyoctadecan-1-yl acetate	15	b,c
84	2-Hydroxyicosan-1-yl acetate	215	b,c,i
12	Unsaturated aldehyde	m/z 81 (100), 41 (35), 67 (28), 83	
		(25), and 194 (1)	
13	Unidentified	m/z 79 (100), 41 (70), 67 (68), 91	
		(65), 55 (60), and 192 (1)	
14	Unidentified	<i>m</i> /z 81 (100), 79 (58), 41 (40), 67 (37), 91 (32), 77 (30), and 192 (2)	
15	Unidentified	m/z 41 (100), 55 (100), 83 (78), 57 (68), 67 (67), 84 (66), 43 (65), and 196 (3)	
32	Unidentified	m/z 55 (100), 41 (66), 69 (55), and 208 (5)	(83), 57 (70), 67 68 (54), 96 (53),

TABLE 9. Continued

No. in Figure 1	Compounds	Quantity (pg/animal)	Analytical methods
37	Unidentified	m/z 55 (100), 82 (88), 41 (86), 69 (70), 57 (69), 67 (60), 96 (54), and 222 (6)	
39	Alkyl phthalate (contaminant)		
40	Unidentified	m/z 67 (100), 81 ( (48), 79 (45), 54 and 252 (0.5)	68), 55 (57), 41 4 (42), 95 (39),
46	Unidentified	m/z 43 (100), 55 ( (75), 69 (70), 8 and 281 (2)	93), 41 (88), 57 3 (62), 66 (51),
47	Unidentified	m/z 55 (100), 41 ( (53), 81 (51), 9 and 251 (2)	85), 67 (61), 69 8 (38), 82 (37),
48	Impure peak		
51	Unidentified	m/z 55 (100), 82 ( (78), 81 (69), 9 and 236 (4)	85), 41 (80), 67 5 (60), 80 (56),
55	Unidentified	m/z 67 (100), 55 ( (72), 81 (70), 6 95 (48), and 28(	98), 41 (82), 82 8 (60), 69 (51), 0 (2)
60	Unidentified	m/z 55 (100), 82 ( (63), 67 (55), 6 57 (48), and 250	(83), 41 (69), 96 9 (53), 43 (49), 0 (8)
64	Unidentified	m/z 55 (100), 41 (68), 67 (66), 69 (64), 81 (53), 83 (48), 82 (45), 95 (41), and 262 (4)	
65	Unidentified	<i>m/z</i> 55 (100), 41 (88), 67 (78), 82 (68), 81 (67), 80 (66), 68 (54), 69 (50), and 280 (2)	
68	Unidentified	m/2 80 (100), 67 (73), 55 (68), 41 (58), 81 (57), 68 (44), 94 (43), 95 (37), and 262 (10)	
76	Unidentified	m/z 55 (100), 41 (82), 67 (70), 81 (53), 68 (47), 95 (46), 82 (38), 43 (36), and 306 (14)	
79	Unidentified	m/2 (56), and 560 (17) m/2 (55 (100), 41 (78), 80 (77), 67 (74), 81 (70), 82 (58), 68 (50), 67 (49), 95 (47), and 308 (3)	
85	Unidentified	m/z 43 (100), 57 (56), 69 (54), 9 82 (35), 71 (33	(66), 55 (60), 83 17 (48), 41 (38), ), and 356 (0.3)

TABLE 9. Continued

a: Identified in previous study; b: low-resolution mass spectrum; c:  $Cl(CH_4)$  mass spectrum; d: Cl(NO) mass spectrum; e: EI mass spectrum of dimethyl disulfide derivative; f: high-resolution mass spectrum; g: <sup>1</sup>H NMR spectrum; h: <sup>13</sup>C NMR spectrum; i: retention time comparison; j: detected using a polar column; k: double bond position(s) uncertain.

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