Chemical Composition of the Nasal Gland Secretion from the Marsh Deer *Odocoileus (Dorcelaphus) dichotomus* (Illiger)

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The secretion of the nasal gland from the marsh deer *(Odocoileus dichotomus)* has shown to be a lipid mixture predominantly containing cholesterol esters, mono- and diester waxes, the latter of which containing 2-hydroxy fatty acids. The alcoholic constituents of the mono- and diester waxes contain considerable amounts of mono-unsaturated homologues, all with double bonds in (ω-9)-position.

In a recently published paper [1] we reported on a nasal gland of *Odocoileus dichotomus* Illiger, the marsh deer, living in the South American swampy steppes between Guayana and Uruguay. The greasy secretion of this gland has shown to be a complex mixture of lipids [1], the composition and structure of which will be presented in this paper.

Material and Methods

The waxy secretion was scraped from the nasal gland from a freshly died animal (♂, Krefelder Zoo) and extracted with $CHCl_3/CH_3OH$ (2:1, v/v, 60 ml) and the mixture diluted with water (20 ml). The hypolayer was evaporated and yielded a greasy material (434 mg) which was separated by column chromatography on SiO_2 (Woelm, 14.5% water content) by eluting with a $C_6H_{12}/C_6H_6/CHCl_3$ gradient. Cholesterol esters and monoester waxes (together 96.6 mg) were eluted with cyclohexane/benzene (9:1; v/v; 100 ml), diester waxes with (9:6; v/v; 75 ml). An intermedial fraction (77.8 mg) and cholesterol (19.2 mg) were eluted with benzene (50 ml) and benzene/chloroform (9:1; v/v; 50 ml), respectively, and more polar compounds (38 mg) with chloroform/methanol (2:1; v/v; 50 ml).

The mixture of cholesterol esters and monoester waxes was separated into methyl esters and alcohols after methanolysis with 5% methanolic HCl. Diester waxes were saponified by 45 min boiling with 1 N methanolic KOH. The resulting acids were esterified with 5% methanolic HCl. Samples of the alcohols were oxidized with CrO_3/AcOH in cyclohexane [2] as well as with IO_4'/MnO_4' as described elsewhere [3], and the resulting acids esterified.

Gas-liquid chromatography (GLC) was performed on polar and unpolar phases using a 3 m glass column with 5% DEGS on Celite and a 10 m glass column with 3% OV 101 on GasChrom Q. Mass spectra were recorded with the Varian-MAT 111 (GNOM) instrument at 80 eV equipped with a glass connection between inlet and ion source.

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Results

After column chromatography of the nasal secretion from *Odocoileus dichotomus* three main lipid fractions could be obtained: cholesterol esters, mono- and diester waxes (Table I).

Table I. Quantitative lipid composition of the nasal secretion from *Odocoileus dichotomus*.

Lipid	% weight
cholesterol esters and monoester waxes (~1:1)	22.3
diester waxes	46.6
unidentified fraction	17.9
cholestrol	4.4
polar lipids	8.8

After esterification of the fraction containing cholesterol esters and monoester waxes with methanolic HCl the alcoholic and acidic fractions showed patterns of homologues as listed in the Tables II and III. Only unbranched alcohols could be detected in the ester wax fraction and all unsaturated alcohols possess the double bond in (ω-9)- position. The fatty acids, however, contain considerable

amounts of monomethyl-substituted components with branches in the middle of the chain.

Table II. Quantitative composition of the alcoholic constituents of the fraction containing cholesterol ester and monoester waxes.

Alcohols saturated	%	Alcohols unsaturated	%
alcohols (total)	(18.6)	alcohols (total)	(25.4)
n-C_{18}	0.7	$C_{24:1(\omega-9)}$	1.0
n-C_{20}	0.3	$C_{25:1(\omega-9)}$	0.5
n-C_{22}	2.0	$C_{26:1(\omega-9)}$	7.5
n-C_{24}	2.8	$C_{28:1(\omega-9)}$	0.6
n-C_{25}	2.0	$C_{30:1(\omega-9)}$	15.8
n-C_{26}	8.3	cholesterol	56.0
n-C_{28}	2.5		

Table III. Quantitative composition of the acidic constituents of the fraction containing cholesterol esters and monoester waxes.

Fatty acid	%	Fatty acid	%
unbranched		branched	
acids (total)	(84.7)	acids (total)	(7.3)
n-C_{14}	1.8	10-C_{14}	0.2
n-C_{15}	7.8	12-C_{16}	5.6
n-C_{16}	48.7	12-C_{18}	1.5
n-C_{17}	5.6	unsaturated	
n-C_{18}	17.0	acids total)	(8.0)
n-C_{20}	3.8	$C_{18:1}$	8.0

The diester waxes are composed of 2-hydroxy fatty acids esterified with unbranched fatty acids and saturated and (ω-9)-mono-unsaturated alcohols, the composition both of which are given in the Tables IV and V.

Table IV. Quantitative composition of the acidic constituents of the diester waxes from the nasal gland secretion from *Odocoileus dichotomus*.

Fatty acid	%	2-Hydroxy fatty acid	%
total	(44.9)	total	(55.1)
n-C_{14}	1.5	2-OH-C_{16}	30.5
n-C_{15}	1.4	2-OH-C_{17}	7.4
n-C_{16}	32.5	2-OH-C_{18}	17.2
n-C_{17}	1.6		
n-C_{18}	6.3		
n-C_{19}	1.6		

All components have been identified by mass spectrometry. The mass spectra of the esters of 2-hydroxy fatty acids showed in accordance to results

Table V. Quantitative composition of the alcoholic constituents of the diester waxes from the nasal gland secretion from *Odocoileus dichotomus*.

Alcohol	%	Alcohol	%
saturated (total)	(63.0)	unsaturated (total)	(37.0)
n-C_{16}	1.4	$C_{24:1(\omega-9)}$	1.4
n-C_{18}	0.8	$C_{25:1(\omega-9)}$	1.1
n-C_{20}	trace	$C_{26:1(\omega-9)}$	6.0
n-C_{21}	0.6	$C_{27:1(\omega-9)}$	1.0
n-C_{22}	0.6	$C_{82:1(\omega-9)}$	5.6
n-C_{23}	1.5	$C_{29:1(\omega-9)}$	1.1
n-C_{24}	11.4	$C_{30:(\omega-9)}$	7.9
n-C_{25}	10.5	$C_{31:1(\omega-9)}$	1.7
n-C_{26}	30.9	$C_{32:1(\omega-9)}$	9.3
n-C_{27}	2.3	$C_{33:1(\omega-9)}$	1.9
n-C_{28}	2.8		
n-C_{29}	0.2		

of Ryhage and Stenhagen [4] a large (M-59)-fragment corresponding to the split between C-1 and C-2, eliminating $-COOCH_3$, and a small but significant (M-104)-fragment resulting from the split between C_3 and C-4 eliminating $(CH_2-CHOH-COOCH_3+H)$ which corresponds furthermore to the ion *m/e* 103. In addition water elimination (M-18) and methanol elimination (M-32) are observed. Furthermore a (M-45)-fragment resulting from elimination of C-2 and C-3 and a (M-78)-fragment due to a loss of water and$HCOOCH_3$ were detected. A spectrum of methyl 2-hydroxyheptadecanoate is given in Fig. 1.

The oxidative cleavage of the alcohols with IO_4'/MnO_4' yielded only one monobasic acid (nonanoic acid) indicating that all monoenoic alcohols possess a (ω-9)-double bond. This corresponds to the pattern of dibasic acids ($D_{15}-D_{24}$) resulting from the oxidation. The mass spectra of the esters of these acids were identified by authentic spectra [5]. They showed small molecule peaks and large (M-31)-, (M-64)- and (M-73)-fragments as well as the fragmentation pattern (M-73) $\xrightarrow{-CH_3OH}$ (M-105) $\xrightarrow{-H_2O}$ (M-123).

Discussion

Although nothing is known about the physiological function of the nasal gland of *Odocoileus dichotomus* it is unlikely from various reasons that the secretion investigated in this paper contributes actively to a pheromonal system as for instance the tarsal gland secretion of Odocoileus hemionus columbianus [6–10]. The location of the gland and the

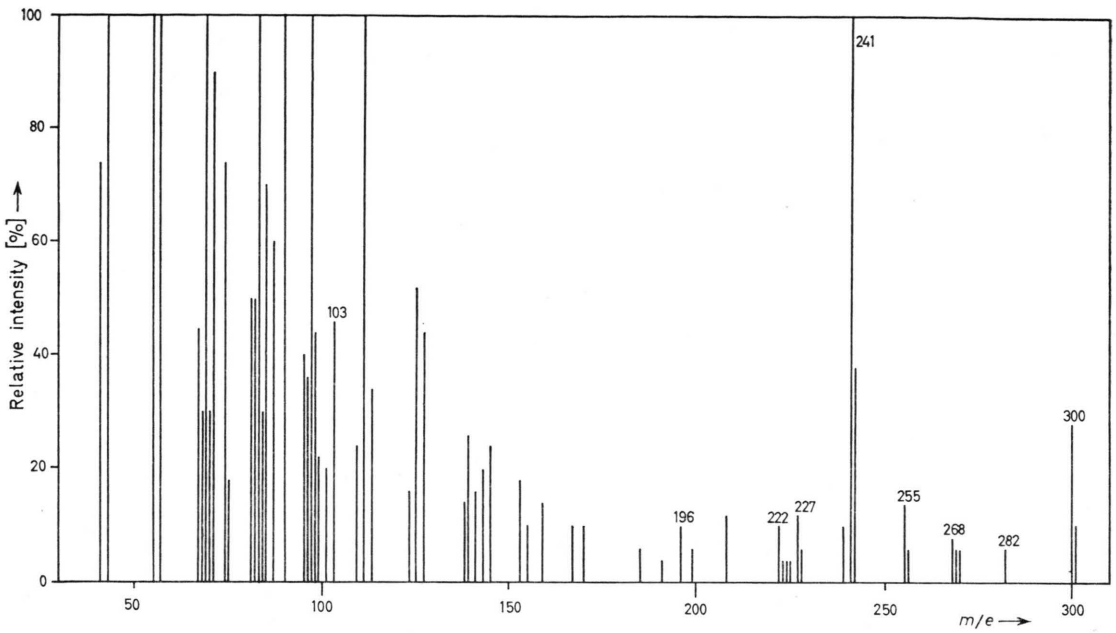

Fig. 1. Mass spectrum of methyl 2-hydroxyheptadecanoate (80 eV) resulting from the diester waxes of the nasal gland secretion from *Odocoileus dichotomus*.

low volatility of the lipids lead us to assume other functions of this secretion which perhaps could be fixing odors, or a contribution to the humidification of the nasal tract, or only protecting function.

2-Hydroxy fatty acids occur in skin surface lipids of the rat [11] and show some but low bacteriostatic properties [12]. They furthermore have been detected as lipid constituents of the nasal salt gland of some birds [13]. The relatively uncommon unsaturated alcohols with double bonds in (ω-9)-position as wax constituents show a certain similarity to the lipids extracted from the Harderian gland of the rat, where monoester waxes, containing alcohols with (ω-9)-double bonds, recently have been observed [14].

[1] J. Jacob and E. von Lehmann, Säugetierk. Mitt. **24** [1976], in press.
[2] J. Jacob, J. Chromatogr. Sci. **13**, 415–422 [1975].
[3] G. Grimmer and J. Jacob, Z. Naturforsch. **24 b**, 565–568 [1969].
[4] R. Ryhage and E. Stenhagen, Ark. Kemi **15**, 545–560 [1960].
[5] R. Ryhage and E. Stenhagen, Ark. Kemi **14**, 497–504 [1959].
[6] R. G. Brownlee, R. M. Silverstein, D. Müller-Schwarze, and A. G. Singer, Nature **221**, 284–285 [1969].
[7] D. Müller-Schwarze, Nature **223**, 525–526 [1969].
[8] D. Müller-Schwarze, Anim. Behav. **20**, 788–797 [1972].
[9] D. Müller-Schwarze, J. Mammalogy **53**, 393–394 [1972].
[10] D. Müller-Schwarze and R. M. Silverstein, Naturw. Umsch. **74**, 88–89 [1974].
[11] T. Nikkari and E. O. A. Haahti, Biochim. Biophys. Acta **164**, 294–305 [1968].
[12] J. Jacob, unpublished results.
[13] K.-A. Karlsson, B. E. Samuelsson, and G. O. Steen, Eur. J. Biochem. **46**, 243–258 [1974].
[14] U. Murawski and U. Jost, Chem. Phys. Lipids **13**, 155–158 [1974].