Mammalian Pheromone Studies, V*. Compounds from the Preorbital Gland of the Grysbok, *Raphicerus melanotis*

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A series of straight chain ketones, saturated, unsaturated, and doubly unsaturated alcohols, saturated and unsaturated formiates, hexanal, formic acid, and a number of long chain fatty acids have been identified in the preorbital secretion of the grysbok, *Raphicerus melanotis.*

The secretion of the preorbital gland of the small neotragine antelope *Raphicerus melanotis*, commonly known as the grysbok, is deposited on twigs [1] by the males in territorial marking. The gland appears to be equally productive in both sexes and the secretions produced by males and females contain the same compounds, albeit in different quantitative ratios. The results of an investigation of the relationship between composition of the preorbital gland secretion, and sex and social status will be communicated elsewhere. In this communication we now wish to report the identification of 34 of the compounds present in the preorbital secretion.

In order to avoid losing volatile material during the evaporative removal of a solvent and thereby distorting the quantitative composition of the secretion, material was collected and introduced into the inlet of the gas chromatograph for GC and GC-MS analysis (SE 30, glass capillary column, 65 m × 0.4 mm) without using a solvent. This was achieved by drawing the secretion into a cooled (ca. −50 °C) glass capillary, sealing one end of the capillary, and introducing it into the inlet through an otherwise stoppered hole in the inlet septum, while keeping the column oven at room temperature. After five minutes the capillary was replaced by a glass stopper and the program started. This procedure gave satisfactory gas chromatograms. The GC-MS analysis, however, produced a total ion current trace (Fig. 1) that showed excessive tailing due to the fact that the gc-ms interface, in which a short length of platinum capillary was used, was kept at as low a temperature as possible; typically at ca. 30 °C below the final column temperature.

The high and low resolution mass spectrometric data were relatively uninformative, but indicated that almost all the constituents of the volatile fraction of the secretion were long chain hydrocarbons and/or alcohols. In order to obtain further spectrometric information, the major constituents of the secretion were isolated by preparative gas chromatography (SE 30, 1.6%, 15 m glass column) for ^1H and ^{13}C NMR analysis and micro-ozonolysis.

From the NMR spectra of a number of representative compounds it immediately became clear that unbranched alcohols and formiates were present in the secretion. Long chain alcohols and formiates exhibit almost identical mass spectra and the presence, in a few cases, of two compounds with apparently identical spectra could thus be ascribed to the presence of these compound types in the secretion. On closer scrutiny, weak (ca. 0.1%) molecular ions were found in both the high and low resolution mass spectra of the formiates. The oxygen containing M-H_2O ion (ca. 0.5%) and the peak at *m/e* 102 (ca. 0.5%, $C_5H_{10}O_2$) were of particular diagnostic value in the identification of those saturated and unsaturated formiates that could not be isolated preparatively.

The location of the double bond in the monounsaturated compounds was established by microozonolysis [2] and gas chromatographic identification of the resulting aldehydes. Assignment of *Z* configuration to the double bond in these compounds was based on the comparison of the ^1H olefinic resonance with resonance patterns simulated with *Z* and *E* coupling constants [3] or on the chemical shift at δ_c ca. 27.3 of the allylic carbon atoms. The ^{13}C resonance of these carbon atoms is expected at δ_c 32.5 for the *E* compounds [4].

The structures assigned to the doubly unsaturated components (1192) and (1383) are based on the formation of hexanal as one of their ozonolysis

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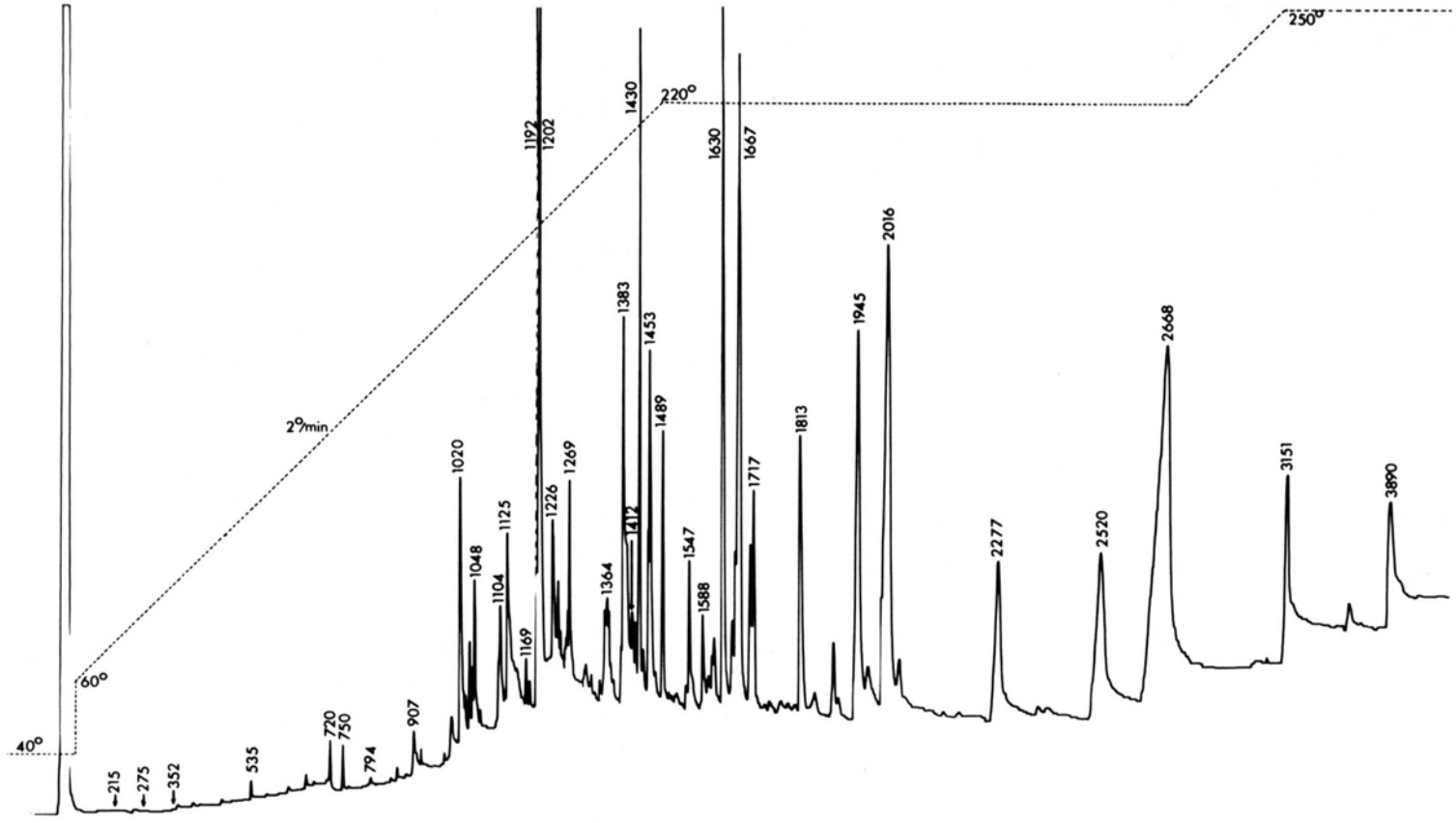

Fig. 1. Gas chromatogram (total ion current, SE 30) of the preorbital secretion of a male grysbok, *Raphicerus melanotis.*

Table I. Compounds identified in the preorbital secretion of the grysbok. (a) Low resolution MS; (b) high resolution MS; (c) ^1H NMR (down to 1 µg sample size); (d) ^{13}C NMR (down to 80 µg sample size); (e) micro-ozonolysis; (f) found with Carbowax 20M as stationary phase.

f	HCOOH	a	2016	OCHO	abcd
215	CHO	a	2277	OCHO	abc
275	O	a	2668	OCHO	abcd
352	O	a	3151	OCHO	ab
f	O	a	3890	OCHO	ab
794	CH_2OH	a	1104	CH_2OH	ab
907	CH_2OH	abc	1202	CH_2OH	abcde
1020	CH_2OH	abc	1453	CH_2OH	abc
1125	CH_2OH	abc	1717	CH_2OH	ab
1226	CH_2OH	abc	1430	OCHO	abcde
1169	OCHO	abc	1630	OCHO	abce
1269	OCHO	ab	1945	OCHO	abce
1364	OCHO	abc	2520	OCHO	abce
1547	OCHO	ab	1192	CH_2OH	abcde
1667	OCHO	abc	1383	CH_2OH	ab
1813	OCHO	abc	f	COOH	a
			1412	COOH	ab
			1588	COOH	ab

products and on the presence of the typical resonance at δ 2.8 of a methylene group adjoined by two double bonds. As in the case of the mono-unsaturated compounds, *Z* configuration was assigned to both these double bonds on the basis of the chemical shift at δ_c 27.23 of the two outer allylic carbon atoms. This assignment was substantiated by the resonance at δ_c 25.46 of a methylene carbon atom adjoined by two double bonds with *Z* configuration [5]. As the preparative isolation of constituents yielded insufficient material in the case of three unsaturated components (1104), (1717), and (1383), the double bonds in these compounds were assumed also to possess *Z* configuration.

Known compounds were identified by comparison of their mass spectra with published data [6, 7]. The compounds identified in the preorbital secretion and the respective analytical methods employed, are given in Table I.

Acknowledgements

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