Journal of Chemical Ecology, Vol. 27, No. 6, 2001

MAMMALIAN EXOCRINE SECRETIONS XV. CONSTITUENTS OF SECRETION OF VENTRAL GLAND OF MALE DWARF HAMSTER, *Phodopus sungorus sungorus*

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(Received January 3, 2000; accepted January 30, 2001)

Abstract—In a study aimed at the chemical characterization of constituents of the ventral gland secretion of the male dwarf hamster, *Phodopus sungorus sungorus*, 48 compounds, including saturated alcohols, saturated and unsaturated ketones, saturated and unsaturated straight-chain carboxylic acids, iso- and anteisocarboxylic acids, 3-phenylpropanoic acid, hydroxyesters, 2-piperidone, and some steroids were identified in the secretion. The position of the double bonds in γ-icosadienyl-γ-butyrolactone and γ-henicosadienyl-γ-butyrolactone, and the position of methylbranching in seven C_{16}–C_{21} saturated ketones could not be established. Several constituents with typically steroidal mass spectra also remained unidentified. The female dwarf hamster's ventral gland either does not produce secretion or produced so little secretion that it was impossible to collect enough material for analysis.

Key Words—*Phodopus sungorus sungorus*, dwarf hamster, mammalian semiochemicals, mammalian pheromones, exocrine secretion, ventral gland secretion.

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INTRODUCTION

The dwarf hamster, *Phodopus sungorus*, also known as the Djungarian striped or hairy-footed hamster, is a small rodent found on different types of steppes characterized by extreme temperature differences such as the dry steppes of Mongolia, Western Siberia, where air temperatures vary between $+30^{\circ}$C in summer and -40°C in winter (Heldmaier, 1975), and in the eastern parts of the Lake Baikal region (Pogosianz and Sokova, 1967), as well as on the steppes of Kazakhstan, Manchuria, and North China (Wynne-Edwards and Lisk, 1984). The dwarf hamster is unusually resistant to low temperatures (Veselofsky and Grundova, 1965). It was found (Heldmaier, 1975) that they tolerated severe cold stress, but were less able to withstand heat.

Phodopus belongs to the family Cricetinae, of which they are one of the smallest species with a body length between 67 and 102 mm (Flint, 1966). Two subspecies of the dwarf hamster are mentioned in the literature, namely *P. s. sungorus* (Pallas), which is found in the western regions of their habitat, and the eastern subspecies *P. s. campbelli* (Thomas). However, the majority of authors make no distinction between the subspecies. A few minor differences between the subspecies have been reported. *P. s. campbelli* is gregarious (Wynne-Edwards and Lisk, 1984), while *P. s. sungorus* appears to be more solitary (Reasner and Johnston, 1988). The seasonal pelage color change from dark grey in summer to white in winter is, for example, also quite pronounced in *P. s. sungorus* (Heldmaier, 1975).

Although their cold resistance is noteworthy, the most unusual characteristic of these hamsters is their highly photoperiodic nature. It seems that rather than hibernate, they have adapted strategies for survival of severe winter conditions by making use of the extreme seasonal changes in photoperiod, which are characteristic of their natural habitat. During short photoperiods ($\leq$8 hr of light), as experienced in the Siberian winter, a reduction in body weight occurs, pelage color changes dramatically, male testicle regression occurs and reproductive ability is diminished (Hoffmann, 1973), sexual development is slowed down (Brackmann, 1977; Yellon and Goldman, 1984), and a reduction in the ventral gland size is also observed (Sunderkötter et al., 1990).

According to Heisler (1984), the olfactory marking behavior in this hamster includes marking with feces, urine, and secretions from the ventral gland, as well as sand-bathing behavior, in which the animal rolls on its back, not necessarily only in sand. The marking behavior of both males and females in neutral areas, in areas where males had previously marked, and in areas where females had previously marked was investigated. The marking frequency was found to be low in both sexes and lower in females than in males. The hamsters were observed to mark the periphery of the test area more frequently than they marked in the nonperipheral areas, and while the males did not mark more frequently in an area that had already

been marked by a male, they did show an increased marking frequency in areas previously marked by females.

Feoktistova (1994) investigated the behavioral responses of adult, sexually experienced males of *P. sungorus* toward different olfactory cues, such as urine, bedding material, and integumentary skin gland secretions of conspecific males and diestrous females. Habituation studies showed that males were able to discriminate between the odors of males and females. The odors of urine, bedding material, and the secretion of the sacculi, located at the opening of the cheek pouches of conspecific males, elicited a high level of midventral gland marking, while the same odors of females resulted in anogenital marking. The low population density, nocturnal activity, and arid habitat of the dwarf hamster are expected to favor marking activity in these animals. *P. s. sungorus* has well developed glands, of which the ventral sebaceous gland (*glandula abdominales*) located along the axis body line before the genitals, is one of the most important. The gland becomes visible at an age of about 4 weeks, and it functions only during the reproductive period. The hamsters use the secretion of this gland for marking their home ranges.

In contrast to the large volume of literature on olfaction in other rodent species, no comprehensive chemical investigation of the constituents of the secretions of the dwarf hamster has so far been undertaken. The aim of the present study is to initiate chemical investigations into the nature of the exocrine secretions in hamsters, specifically in *P. s. sungorus*, in order to provide information that could lead to new avenues of investigation of chemical communication systems in hamsters. It is hoped, for example, that the compounds found in the secretions will generate ideas as to their possible origin and/or purpose, and will enable biologists to use them in behavioral studies. Using compounds present in the hamster secretions in research on rodent olfactory receptors and behavior, makes much more sense than using arbitrarily selected compounds totally unrelated to substances involved in the semiochemical communication of these animals.

METHODS AND MATERIALS

General. Dichloromethane (1 ml) (Merck, residue analysis grade), was concentrated to 10 μl, analyzed for impurities, and found to be pure enough for extraction of small quantities of semiochemicals. Syringes were cleaned with this solvent. Glassware used for the collection and extraction of samples was washed, rinsed with distilled water, heated in an annealing oven at 500°C for at least 30 min, and cooled immediately prior to use to remove all traces of adsorbed organic material.

Analytical Methods. Gas chromatographic analyses were carried out with a Carlo Erba 5300 gas chromatograph equipped with a flame ionization detector, Grob split-splitless injector, and glass columns manufactured by the Laboratory for Ecological Chemistry, University of Stellenbosch. Glass capillary columns, coated

with a 0.25-μm film of the apolar stationary phase PS-089-OH, which is a silanol-terminated 95% dimethyl–5%-diphenyl siloxane copolymer, were used throughout the study. Helium was used as carrier gas at a linear velocity of 28.6 cm/sec at 40°C. The detector and injector were used at 280°C and 220°C, respectively. Samples were injected in the split mode (split ratio 6 : 1) and an oven temperature of approximately 30°C. The oven was heated ballistically to 40°C and programmed at 2°/min from 40° to 260°C (hold). Gas chromatographic retention time comparison of the constituents of the secretion with the commercially available and synthesized reference compounds was done by coinjection of the reference compound and glandular extract to determine whether the component under investigation was enriched by the reference compound.

Quantitative gas chromatographic analyses were done with the same instrument and column, and data acquisition with Borwin Intuitive Chromatography Software (JMBS Developments, 38600 Fontaine, France) using hexadecanoic acid as external standard. Ventral gland secretion (4.8 mg) was collected, extracted with dichloromethane as described below, and the extract concentrated to 20 μl of which 1-μl quantities were injected for analysis.

A Carlo Erba QMD 1000 GC-MS system was used to record electron impact (EI) mass spectra (70 eV) employing the GC parameters specified above. The interface temperature was maintained at 250°C. The ion source temperature was 200°C, and the source pressure varied between *ca.* 2×10^{-5} torr at a column temperature of 40°C, decreasing to 1×10^{-5} torr towards the end of the temperature program at a column temperature of 260°C. A scan rate of 0.9 scan/sec, with an interval of 0.1 sec between scans, was employed. Chemical ionization (CI) mass spectra with methane as reactant gas were recorded on a Finnigan 4500 quadrupole GC-MS system. The interface temperature was 220°C, and spectra were recorded at 60 eV and at a source temperature of 150°C. Under these conditions the pressure in the source was approximately 7×10^{-5} torr. Spectra were recorded at a rate of 2 scans/sec. Accurate mass measurements of the synthetic compounds were done on an AMD 604 magnetic sector mass spectrometer. The spectra were recorded at an ionization potential of 70.7 eV and a source temperature of 192°C. The resolving power was 4000, and a scan rate of 1 scan/sec was used, with a recovery time of 0.5 sec. A sampling frequency of 100 kHz was used, and the source vacuum was 1.4×10^{-5} torr. In the case of samples introduced on the probe, the probe was heated from 20° to 350°C.

^{1}H and ^{13}C NMR spectra of synthesized compounds were obtained on a Varian VXR-300 spectrometer at 299.9 MHz and 75.42 MHz, respectively. The ^{13}C spectra were obtained by using a 45° pulse angle and a pulse repetition time of 0.8 sec. Chloroform-d_1 (Merck, 99.8% isotopic purity) was used as solvent and tetramethylsilane (TMS) as internal reference.

Determination of Double Bond Positions of Constituents. Dimethyl disulfide (DMDS) derivatives were prepared from the unsaturated constituents of

the secretion according to the procedure described by Vincenti et al. (1987). A dichloromethane extract of the secretion was concentrated in a Reacti-Vial using a slow stream of purified nitrogen. The residual material was dissolved in carbon disulfide (50 μl) and treated with an excess (5-μl) of a solution of iodine in ether (60 mg/ml) and 50 μl of dimethyl disulfide. The Reacti-Vial was sealed and the reaction mixture heated in an oven at 60°C for 40 hr; then the iodine was reduced with aqueous sodium thiosulfate solution (5%). Separation of the aqueous and organic layers was facilitated by centrifuging the reaction mixture at 3000 rpm. The organic layer was transferred to another vial and concentrated to 5 μl for GC-MS analysis.

Sample Collection and Preparation. Djungarian dwarf hamsters, *P. s. sungorus*, were bred from stock obtained from a breeding colony of the Zoological Institute at the University of Bonn. All adult animals were kept indoors in separate wire cages (36 × 25 × 23 cm) with an exercise wheel, except when males and females were put together for breeding purposes. The animals were kept at a constant temperature of 22°C in a laboratory free from artificial odors. To simulate summer conditions, the animals were exposed to natural light during daytime (Stellenbosch 33°50′ southern latitude) and, in addition, fluorescent lights were left switched on permanently resulting in a minimum light intensity of 200 lux during the nighttime. Each hamster was supplied with half a coconut shell that served as a hut into which it could withdraw into almost total darkness. To avoid contamination of the secretions with plant volatiles, shavings from old, dry poplar logs were provided for bedding material. When breeding, animals were also provided with cellulose tissues. All bedding and cellulose materials were renewed at weekly intervals. All hamsters were provided *ad libitum* with water and food containing sunflower seeds. Twice a week they were supplied with slices of apple, carrot, and lettuce. No problems were experienced with maintaining and breeding the hamsters under these conditions.

Teflon stoppers and lengths of stainless-steel wire (15 × 0.4 mm) were washed with detergent solution; rinsed with distilled water, methanol, and dichloromethane, and dried in an oven at 110°C. Using precleaned tweezers, the stainless-steel wires were inserted into the tapered end of the Teflon stoppers. The other ends of each of the wires were bent to form a small eyelet having a diameter of approximately 1 mm with which the secretion could be removed from the gland. Ventral gland secretion was collected individually from mature males in their summer state on a weekly basis. Although a hairless secretory area was clearly visible on females in this state, they did not produce enough substance for collection. The waxy secretion was collected by scooping it from the glandular area with a precleaned stainless-steel eyelet. The Teflon stoppers were inserted into the flared ends of small glass vials to provide an air-tight stopper for the vials The samples were stored at −20°C for future use.

For analysis, initially the collected secretion was scraped off the wire loop and transferred to a Reacti-Vial containing dichloromethane. However, a substantial

proportion of the sample was lost in this process. To minimize the loss of sample, the stainless steel eyelet containing the sample was therefore cut off and allowed to fall into a Reacti-Vial containing 30 μl of dichloromethane. The material was sonicated for 20 min, and then the light brown, turbid extract was centrifuged at 2500 rpm for 15 min in order to separate the extract into a clear dichloromethane extract and a turbid supernatant layer. The bottom layer was removed from underneath the supernatant layer with a 100-μl syringe and transferred to a clean Reacti-Vial in which the extract was concentrated in an inert atmosphere to a concentration suitable for GC and GC-MS analysis.

Reference Compounds. In addition to authentic commercially available reference compounds, the following compounds were synthesized for retention time and mass spectral comparison with constituents of the ventral secretion.

2-Heptadecanol (**17**); Figure 1 was prepared by $NaBH_4$ reduction of 2-heptadecanone. HR-MS: *m/z* $(M\text{-}H_2O)^+$ 238.267, calcd. for $C_{17}H_{34}$ 238.266. ^{13}C NMR ($CDCl_3$): $\delta = 68.20$ (d, C-2), 39.41 (t, C-3), 31.95 (t, C-15), 29.4–29.8 (t, C-6–C-14), 29.38 (t, C-5), 25.80 (t, C-4), 23.49 (q, C-1), 22.71 (t, C-16), 14.12 (q, C-17).

(*Z*)-6-Heptadecen-2-one, (*Z*)-8-heptadecen-2-one (**13**), (*Z*)-6-nonadecen-2-one, (*Z*)-8-nonadecen-2-one (**28**), (*Z*)-10-nonadecen-2-one, (*Z*)-12-nonadecen-2-one (**29**), and their *E* isomers were prepared by alkylation of ethyl acetoacetate with the appropriate (*E*)- and (*Z*)-alkenyl bromides, hydrolysis of the condensation product, and decarboxylation of the resulting β-ketoacid according to the following procedure described for the preparation of (*Z*)-8-nonadecen-2-one.

A solution of tetrabromomethane (41.5 mg, 0.125 mmol) in dry acetonitrile (230 μl) was added to a solution of (*Z*)-5-hexadecen-1-ol (30 mg, 0.125 mmol) (Pherobank, Wageningen, The Netherlands) and triphenylphosphine (32.8 mg, 0.125 mmol) in acetonitrile (230 μl) in a 3-ml Reacti-Vial at room temperature and the reaction mixture stirred magnetically at room temperature for 24 hr. The resulting bromide and traces of unchanged starting compounds were extracted with *n*-pentane from the acetonitrile by magnetically stirring the reaction mixture with the *n*-pentane (300 μl), centrifuging the mixture at 1000 rpm for a few minutes and removing the supernatant pentane layer with a 500-μl syringe. The extraction was repeated five times. The reaction product was purified by bulb-to-bulb distillation to give (*Z*)-1-bromohexadec-5-ene, 36 mg (95%) bp 102 (air bath)/7.5×10^{-4} mm Hg. The alkenyl bromide, containing less than 0.1% of the unchanged 5-hexadecen-1-ol as the only impurity (GC-MS), was used to alkylate ethyl acetoacetate. Ethyl acetoacetate (1.35 g, 10.4 mmol) was deprotonated by adding it to a solution of sodium ethoxide (10.4 mmol) in absolute ethanol (50 ml). The resulting anion was alkylated by magnetically stirring a sample (600 μl) of this solution in a 3-ml Reacti-Vial with (*Z*)-1-bromohexadec-5-ene (36 mg, 0.119 mmol) at 90°C for 11 hr during which time a precipitate of NaBr was formed. The ethanol was evaporated at room temperature by using a slow stream of purified

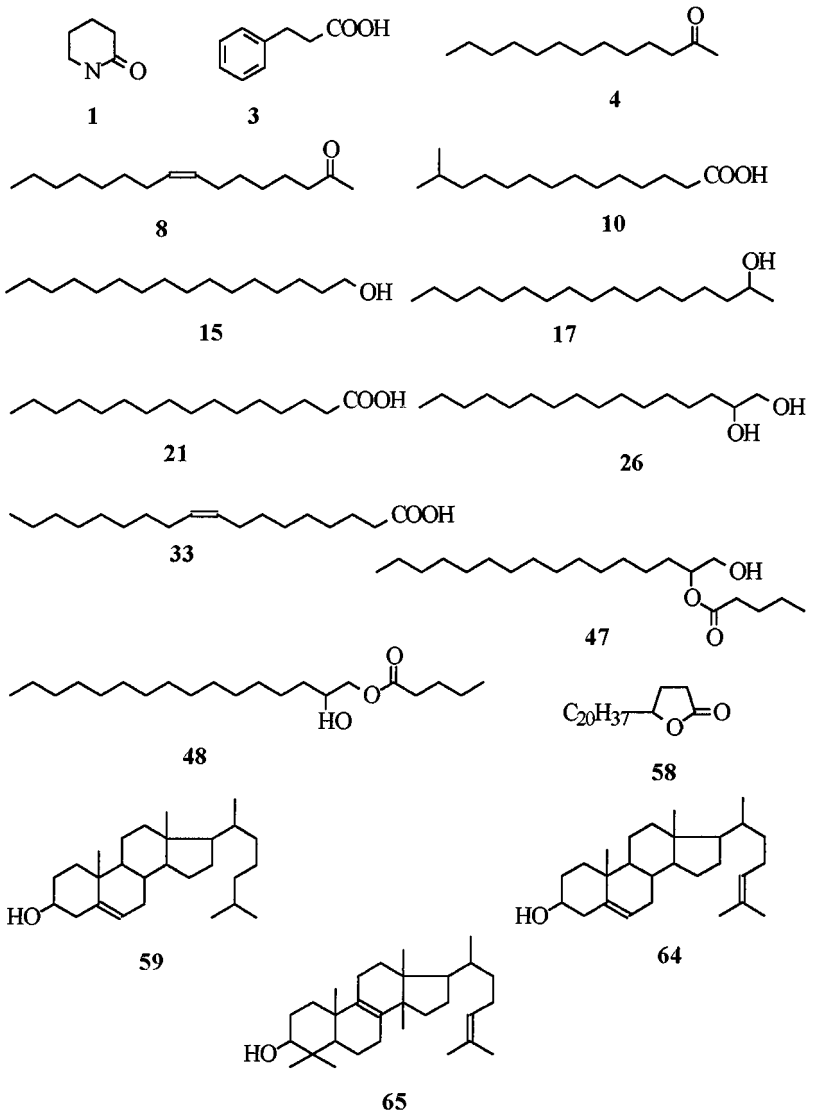

FIG. 1. Structures of representative examples of the compounds identified in the ventral secretion of the male dwarf hamster, *Phodopus sungorus sungorus.*

argon, after which the ethyl ester was hydrolyzed by stirring the residue in the Reacti-Vial with NaOH solution (1.5 M, 200 μl, 0.3 mmol) at room temperature for 24 hr. The unsaponified material and unchanged bromide were extracted twice with *n*-pentane (300 μl) by stirring the reaction product with the solvent, centrifuging the mixture at 1000 rpm, and removing the supernatant organic layer with a 500-μl syringe. The saponified material was acidified with H_2SO_4 (1.5 M) and the resulting ketoacid decarboxylated by heating the reaction mixture at 90°C until no further formation of gas bubbles in the Reacti-Vial (closed with a screw cap) could be observed. The organic material was extracted three times with *n*-pentane and washed free from H_2SO_4 with distilled water. Slow evaporation of the solvent gave (*Z*)-8-nonadecen-2-one (**28**) as a colorless oil (3.8 mg) containing 2.5% of the *E* isomer (GC-MS). ^{13}C NMR ($CDCl_3$): $\delta = 209.24$ (s, C-2), 130.22 (d, C-9), 129.48 (d, C-8), 43.78 (t, C-3), 31.93 (t, C-17), 29.85 (q, C-1), 29.77–28.83 (t, C-5, C-6, C-11–C-16) 27.25 (t, C-7)*, 27.03 (t, C-10)*, 23.79 (t, C-4), 22.69 (t, C-18), 14.11 (q, C-19) (* assignments interchangeable).

1-Hydroxyhexadec-2-yl pentanoate (**47**) and 2-Hydroxyhexadec-1-yl pentanoate (**48**) were prepared by the Al_2O_3-catalyzed reaction of 1,2-epoxyhexadecane with pentanoic acid as described by Burger et al. (1999a). The two isomers were formed in a ratio of 3 : 2. HR-MS (mixture of the two isomers): *m/z* M^+ 342.309, calcd. for $C_{21}H_{42}O_3$ 342.313. *Major component*: ^{13}C NMR ($CDCl_3$): $\delta = 174.37$(s, C-1), 75.50 (d, C-2′), 64.99 (t, C-1′), 34.30 (t, C-2), 31.95 (t, C-14′), 30.56 (t, C-3′), 29.4–29.7 (t, C-5′–C-13′), 27.16 (t, C-3), 25.33 (t, C-4′) 22.72 (t, C-15′), 22.28 (t, C-4), 14.13 (q, C-16′), 13.72 (q, C-5). *Minor component*: ^{13}C NMR ($CDCl_3$): $\delta = 174.05$ (s, C-1), 70.10 (d, C-2′), 68.58 (t, C-1′), 33.96 (t, C-2), 33.40 (t, C-3′), 31.95 (t, C-14′), 29.4–29.7 (t, C-5′–C-12′), 29.38 (t, C-13′), 27.05 (t, C-3), 25.39 (t, C-4′), 22.72 (t, C-15′), 22.28 (t, C-4), 14.13 (q, C-16′), 13.72 (q, C-5).

2-Piperidone (**1**). Cyclopentanone oxime was dried over phosphorus pentoxide and subjected to Beckmann rearrangement in concentrated H_2SO_4 to give 2-piperidone (δ-valerolactam) (Becker et al., 1973). HR-MS: *m/z* M^+ 99.066, calcd. for C_5H_9NO 99.068. ^{13}C NMR ($CDCl_3$): $\delta = 172.65$ (s, C-1), 42.20 (t, C-5), 31.51 (t, C-2), 22.28 (t, C-4), 20.88 (t, C-3).

RESULTS AND DISCUSSION

A typical total ion chromatogram of an extract of the ventral secretion of a male dwarf hamster is given in Figure 2 and the compounds identified in the secretion are listed in Table 1. Mass spectral data are given for only a few typical examples of the different compound classes present in the secretion, because the spectra of compounds within each class are quite similar and the spectral data of these typically long-chain compounds contain relatively little structural information. The structures of representative examples of each compound type are given

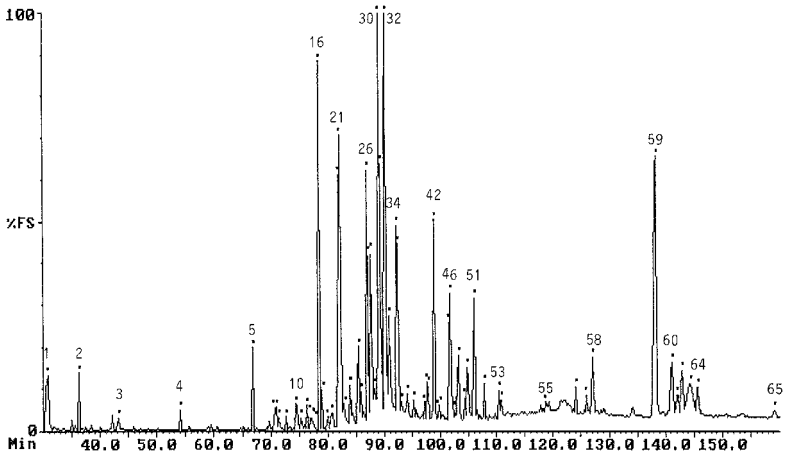

FIG. 2. Total ion chromatogram of an extract of the ventral secretion of a male dwarf hamster, *Phodopus sungorus sungorus.*

in Figure 1. Tentative identification of the constituents of the secretion was based on low-resolution EI mass spectra and CI mass spectral information obtained with methane as reactant gas. Final confirmation of many of the proposed structures was obtained by co-injection of an extract of the secretion with authentic synthetic material.

It is not possible to determine unequivocally the position of double bonds in unsaturated ketones from the low-resolution mass spectral data of their dimethyl disulfide (DMDS) adducts, because one CO and two CH_2 groups are isobaric, so that the DMDS derivative of one alken-2-one can give rise to two diagnostic ions with the same nominal masses (although having different accurate masses) as those from another isomeric DMDS derivative. Both the *E* and *Z* isomers of each of the pairs of alken-2-ones had therefore to be synthesized for retention-time comparison. There appeared to be two series of unsaturated ketones present in the secretion, one with the double bond in the 8 position and another one with the double bond equidistant from the methyl terminal end of the ketones. Although the position of the double bond of the unsaturated ketone **19** could not be determined, it is quite likely that it belongs to the first of these series and could therefore be (*Z*)-8-octadecen-2-one.

Although several presumably methyl-branched 2-alkanones having spectra similar to those of the unbranched ketones are present in the secretion, these compounds are present in such low concentrations that it was impossible to determine the position of the methyl branching from the available mass spectral data. The molecular masses of some of these ketones could be established from

TABLE 1. COMPOUNDS IDENTIFIED IN VENTRAL SECRETION OF DWARF HAMSTER

Peak (Figure 1)	Compound	EI Mass spectral data, *m/z* (%)	Quantity (ng/animal)
15	1-Hexadecanol[a,c]		0.04
17	2-Heptadecanol[a–c]		0.3
31	2-Nonadecanol[a–c]	266(0.4), 238(0.5), 227(0.4), 181(0.5), 168(0.5), 154(0.8), 140(1), 125(1), 111(7), 97(18), 83(19), 7(15), 69(18), 57(33), 55(30), 45(100), 43(47)	0.5
43	2-Henicosanol[a,d]		0.05
26	Hexadecane-1,2-diol[a–c]	227(15), 125(7), 117(6), 111(24), 97(58), 83(73), 71(38), 69(75), 61(22), 57(82), 55(88), 43(100), 41(75)	1.3
32	Heptadecane-1,2-diol[a–c]		7.1
4	2-Tridecanone[a,b,c,e]		0.1
5	2-Pentadecanone[a–d]		0.4
9	2-Hexadecanone[a–d]		0.05
16	2-Heptadecanone[a–d]	254(2), 239(1), 196(2), 194(2), 152(1), 138(1), 127(3), 96(7), 85(15), 71(44), 59(68), 58(100), 55(25), 43(97), 41(30)	0.2
23	2-Octadecanone[a,b,d,e]		0.3
30	2-Nonadecanone[a,d]		4.9
37	2-Icosanone[a,b,d]		0.1
42	2-Henicosanone[a,b,c]		1.2
52	2-Tricosanone[a,b,d]		0.2
6	Branched 2-hexadecanone[a,b,d]		0.1
12	Branched 2-heptadecanone[a,b,d]		0.2
20	Branched 2-octadecanone[a,b,d]		1.0
27	Branched 2-nonadecanone[a,b,d]	282(1), 224(0.1), 220(1), 96(5), 85(10), 71(38), 59(72), 58(100), 55(22), 43(100), 41(25)	0.3
35	Branched 2-icosanone[a,b,d]		2.2
39	Branched 2-henicosanone[a,b,d]		0.1
36	Branched 2-icosanone[a,b,d]		0.3
8	(*Z*)-8-Hexadecen-2-one[a,b,d]		0.06
13	(*Z*)-8-Heptadecen-2-one[a–d]		0.02
14	(*Z*)-10-Heptadecen-2-one[a,b,d]		0.05
19	(*Z*)-8-Octadecen-2-one[a,b, f]		0.09
22	(*Z*)-11-Octadecen-2-one[a,b,d]		0.07
28	(*Z*)-8-Nonadecen-2-one[a–d,g]	280(0.2), 265(0.1), 262(0.2), 222(1), 198(2), 184(1), 152(2), 135(4), 125(10), 111(8), 97(17), 96(15), 82(19), 81(18), 71(33), 58(25), 55(43), 43(100), 41(39)	0.7

TABLE 1. (*Continued*)

Peak (Figure 1)	Compound	EI Mass spectral data, *m/z* (%)	Quantity (ng/animal)
29	(*Z*)-12-Nonadecen-2-one[a−d,g]		0.6
40	(*Z*)-8-Henicosen-2-one[a,b,d,g]		0.2
41	(*Z*)-14-Henicosen-2-one[a,b,d,g]		0.2
21	Hexadecanoic acid[a−c]		4.9
34	Octadecanoic acid[a−d]		2.2
45	Icosanoic acid[a,c,d]	312(6), 269(3), 213(3), 185(4), 171(3), 157(1), 143(2), 129(20), 115(7), 97(18), 83(23), 73(65), 71(33), 61(20), 60(65), 57(69), 55(68), 43(100), 41(58)	0.5
53	Docosanoic acid[a−d]		0.2
55	Tetracosanoic acid[a,c,d]		0.1
10	13-Methyltetradecanoic acid[a−d]		0.3
18	14-Methylpentadecanoic acid[a,c,d]		0.1
24	15-Methylhexadecanoic acid[a−d]	270(4), 227(6), 125(4), 171(3), 157(1), 143(2), 129(16), 115(6), 97(15), 83(20), 73(61), 71(26), 69(35), 61(15), 60(60), 57(58), 55(59), 43(100), 41(67)	0.6
38	17-Methyloctadecanoic acid[a−d]		0.1
44	18-Methylnonadecanoic acid[a,c,d]		0.1
49	19-Methylicosanoic acid[a−d]		0.2
7	11-Methyltridecanoic acid[a−d]		0.2
25	14-Methylhexadecanoic acid[a−d]	270(4), 241(2), 227(3), 213(3), 185(4), 171(4), 157(3), 139(5), 129(16), 111(10), 97(23), 83(28), 73(55), 71(35), 69(44), 61(10), 60(55), 57(87), 55(98), 43(100), 41(86)	0.1
33	(*Z*)-9-Octadecenoic acid[a−e,g]	282(0.4), 264(2), 256(0.5), 227(0.7), 222(1), 213(1.5), 151(2), 137(3), 123(5), 111(10), 97(30), 83(42), 73(27), 69(65), 67(33), 60(25), 57(42), 55(100), 43(80), 41(88)	0.8
3	3-Phenylpropanoic acid[a,c,e]	150(27), 131(3), 104(44), 91(100), 79(10), 118(20), 77(24), 65(17), 51(20), 39(13)	0.2

(*Continued*)

TABLE 1. (*Continued*)

Peak (Figure 1)	Compound	EI Mass spectral data, *m/z* (%)	Quantity (ng/animal)
47	1-Hydroxyhexadec-2-yl pentanoate[a−c,e]		0.2
48	2-Hydroxyhexadec-1-yl pentanoate[a−c,e]		0.4
50	1-Hydroxyheptadec-2-yl pentanoate[a,b,e]	255(0.5), 227(2), 157(0.5), 116(8), 101(10), 85(100), 69(13), 57(48), 55(23), 48(48), 41(25)	0.5
51	2-Hydroxyheptadec-1-yl pentanoate[a,b,e]	323(0.5), 241(2), 145(5), 116(25), 101(45), 85(100), 69(20), 57(58), 55(24), 43(52), 41(33)	0.8
54	2-Hydroxyicos-1-yl acetate[a,c,e]		0.1
58	4-(Hexadecadien-1-yl)-butanolide[a,e,f,h]		0.8
62	4-(Heptadecadien-1-yl)-butanolide[a,e,f,h]	376(1), 358(0.5), 332(0.5), 221(1), 166(1), 151(2), 125(5), 111(16), 97(37), 85(100), 83(42), 69(50), 57(72), 55(75), 43(89), 41(53)	0.7
1	2-Piperidone[a−c,e]	99(100), 70(30), 58(7), 56(15), 55(38), 43(65), 42(78), 41(59), 30(89)	1.0
2	Unidentified[a,b]		0.3
59	Cholesterol[a−c,e]		5.6
56	Unidentified[a]		0.3
57	Unidentified[a]		0.4
60	Unidentified[a]		0.2
61	Unidentified[a]		1.0
63	Unidentified[a]		1.3
64	Desmosterol[a,c,e]		0.5
65	Lanosterol[a,e,i]		0.2
46	Unidentified[a,b]		0.9

[a] Low resolution EI mass spectral data.
[b] $Cl(CH_4)$ mass spectral data.
[c] Retention time comparison.
[d] Relative retention time interval comparison.
[e] Published data.
[f] Position of double bond uncertain.
[g] EI mass spectral data on DMDS derivative.
[h] Configuration of double bond uncertain.
[i] Tentative identification.

their $Cl(CH_4)$ mass spectra. The secretion contains unbranched as well as iso- and anteiso-branched fatty acids and it is, therefore, quite likely that most of the branched 2-alkanones could also be the iso ketones, while the unidentified ketone **36** could be 18-methyl-2-nondecanone. The retention-time increments observed

for the branched and unbranched carboxylic acids, for example, those between the C_{20} and C_{17} acids, correlate very well with the increments between the branched and unbranched ketones and therefore appear to confirm this assumption. Unfortunately, it was not possible to determine the position of the double bonds in the two γ-lactones **58** and **62**. As can be expected, their mass spectra do not contain the required structural information and their DMDS derivatives could not be found in the total ion chromatogram of a sample of the secretion subjected to DMDS derivatization, probably because the high masses of the derivatives precluded their elution from the capillary column.

According to its Cl(CH_4) mass spectrum, constituent **2** has a molecular mass of 166, and the compound apparently has an acetyl function. Its mass spectrum is similar to that of 1-acetylhydrindane, which also has a molecular mass of 166. However, the mass spectrum of this constituent has a prominent ion at *m/z* 109 (23%), which is relatively weak in the NBS library spectrum of 1-acetylhydrindane. Furthermore, the mass spectrum of the constituent **2** does not have an ion at *m/z* 148 which is present in the library spectrum. This constituent is, therefore, listed as unidentified in Table 1.

All of the constituents eluting in the high-molecular-weight range of the total ion chromatogram appear to be steroids. Some of these compounds remained unidentified in the present investigation and will be subjected to further scrutiny in future research on the dwarf hamster, because of their possible involvement in the chemical ecology of the animal.

Varying quantities of ventral secretion were collected from individual males. Table 1 contains quantitative data obtained from a rather large quantity (4.8 mg) of secretion collected from one male animal. These data are included merely to serve as a guideline for biologists planning behavioral studies on the dwarf hamster. The variation in the quantity of ventral secretion collected from individual animals, the probably incomplete extraction of, especially, the more polar compounds with a small volume of dichloromethane, and the fact that different samples of the secretion possibly contain different concentrations of moisture that is not detectable by FID are considered to be responsible for a larger factor of uncertainty than the use of an external standard and the unaccurate determination of the volume to which the sample was finally concentrated.

Carboxylic acids have been identified in many mammalian exocrine secretions. The longer-chain acids seem to be less common, especially in secretions investigated during the 1960s and 1970s, possibly because long-chain acids did not elute from some of the stationary phases and packed columns used during the earlier years of gas chromatography, or eluted as such broad peaks as to be almost undistinguishable from the baseline. Fatty acids were found in the anal sac secretions of many carnivores (Albone, 1984a) and in many other mammalian secretions. The preputial gland secretion of the musk rat, *Ondatra zibethica*, for example, contains a number of saturated, monounsaturated, and diunsaturated,

C_{12}–C_{20} fatty acids (Ritter et al., 1982). Fatty acids are also found in human vaginal secretions (Huggins and Preti, 1976) and in the interdigital secretion of the reindeer, *Rangifer tarandus* (Brundin et al., 1978). Many of the short-chain acids found in these secretions are iso- or anteiso-branched. 3-Phenylpropanoic acid **3** was identified, among others, in the anal sac secretion of the red fox, *Vulpes vulpes*, and the lion, *Panthera leo* (Albone and Eglinton, 1974), and in the urine of coyote, *Canis latrans* (Murphy et al., 1978).

Long-chain carboxylic acids (C_{15}–C_{25}) were found in the occipital secretion of the bactrian camel, *Camelus bactrianus*, together with some shorter-chain acids (Ayorinde et al., 1982). Long-chain fatty acids are particularly abundant in the interdigital secretions of bontebok, *Damaliscus dorcas dorcas*, and blesbok, *D. d. phillipsi*, containing the saturated unbranched C_6–C_8, C_{10}, C_{12}, C_{14}–C_{18}, and C_{20} fatty acids (Burger et al., 1999a). With the exception of the C_7, C_8, C_{10}, and C_{20} homologs, these acids are also present in the preorbital gland secretions of these two subspecies, which also contain the C_{19} acid (Burger et al., 1999b). The interdigital secretions of these two subspecies also contain oleic acid (*Z*9-C_{18}). The preorbital secretion of the grysbok, *Raphicerus melanotis*, contains the C_{14}–C_{18} and C_{20} fatty acids (Burger et al., 1996) and that of the steenbok, *R. campestris*, C_8, C_{10}, C_{12}, C_{14}–C_{16}, C_{18}, C_{20}, and unsaturated fatty acids (Burger et al., 1999c). Only the three C_{16}–C_{18} fatty acids are present in the preorbital secretion of the oribi, *Ourebia ourebi* (Mo et al., 1995) and the C_8, C_{10}, C_{12}, C_{14}–C_{16}, C_{18}, C_{20} fatty acids and several unsaturated analogs in the preorbital secretion of the grey duiker, *Sylvicapra grimmia* (Burger et al., 1990).

With a few exceptions, long-chain alcohols have not been found in many mammalian exocrine secretions. The identification of the C_{12}, C_{14}, C_{16}, and C_{18} alkanols in human vaginal secretions (Huggins and Preti, 1976) is one of these examples. Of the four long-chain C_{16}, C_{17}, C_{19}, and C_{21} alkanols (**15**, **17**, **31**, and **43**) present in the ventral secretion of the dwarf hamster, the C_{16} and C_{21} alkanols have been identified in the interdigital secretion of the bontebok and blesbok (Burger et al., 1999a), and the C_{16} alkanol in the preorbital secretion of the steenbok (Burger et al., 1999c), as well as in the tarsal volatiles of the reindeer, *Rangifer tarandus tarandus* (Andersson et al.,1975). Although the preorbital secretion of the grysbok contains at least 22 alkanols, alkenols, and alkadienols (Burger et al., 1996), the four alkanols found in the ventral secretion of the dwarf hamster are not among them.

Ketones are reasonably common in mammalian exocrine secretions, and Albone (1984b) has cited several examples of secretions containing a few short- to medium-chain ketones. Jemiolo et al. (1987) have found several saturated, unsaturated, and methyl-branched unsaturated ketones in the urine of the house mouse, *Mus musculus*. The ventral secretion of the dwarf hamster, however, seems to be unique in that it contains at least 25 alkan-2-ones and alken-2-ones from C_{13} to C_{23}. Only a few of the shorter-chain members of the group of ketones present in

the ventral secretion of the dwarf hamster were found in other animals, for example, 2-pentadecanone (**5**) and 2-heptadecanone (**16**) in the perineal secretion of the guinea pigs, *Cavia aperea* and *C. porcellus* (Wellington et al., 1979) and pentadecan-2-one in the dorsal secretion of the springbok, *Antidorcas marsupialis*, (Burger et al., 1981).

Unfortunately, the position of the double bonds in the two monounsaturated γ-lactones (**58** and **62**) could not be determined. One is immediately reminded of a similar compound, (*Z*)-6-dodecen-4-olide, that was identified as a component of the extract of the male tarsal hair tuft of the black-tailed deer, *Odocoileus hemionus columbianus* (Brownlee et al., 1969) and later shown to originate from the urine of the animal (Müller-Schwarze et al., 1978). The same compound was identified in the interdigital secretions of the bontebok and blesbok, secretions that also contain the δ-lactone, dodecan-5-olide (Burger et al., 1999a). The absolute configuration of this compound in the bontebok has not yet been established. Hexadecan-5-olide and its C_{17} and C_{18} homologs are present in the preorbital secretion of the bontebok and blesbok (Burger et al., 1999b). Dodecan-4-olide has been found in the occipital secretion of the bactrian camel (Ayorinde et al., 1982), the γ- and δ-lactones, pentan-4-olide, hexan-4-olide, and hexan-5-olide, have been identified in human urine (Zlatkis and Liebich, 1971); and the C_8–C_{12} γ-lactones in human skin lipids (Labows et al.,1979). The C_{15}–C_{18} and C_{20} alkan-4-olides and C_{16} alkan-5-olide were identified in the preorbital secretion of the grysbok (Burger et al., 1996) and the C_{16} and C_{18} alkan-4-olides in the preorbital secretion of the grey duiker (Burger et al., 1990).

Albone (1984c) discussed the possibility that 2-piperidone identified in the anal sac secretions of the dog, *Canis familiaris*, coyote, *C. latrans*, and mink, *Mustela vison*, could possibly be formed by the elimination of water from the precursor 5-aminovaleric acid that is formed by fermentation processes in the anal sac (Albone et al., 1976). 2-Piperidone has also been identified in human vaginal secretions (Huggins and Preti, 1976).

The ventral secretion of the dwarf hamster contains five long-chain hydroxyesters; **47**, **48**, **50**, **51**, and **54**. Although these hydroxyesters are not accompanied by long-chain epoxides in the ventral and other secretions, it is nevertheless accepted as a working hypothesis that they are formed through nucleophilic substitution of the corresponding epoxides by the corresponding carboxylic acids. The preorbital secretion of the grysbok contains 2-hydroxyoctadecan-1-yl acetate and 2-hydroxyicosan-1-yl acetate (Burger et al., 1996). The related steenbok's preorbital secretion contains 17 of these hydroxyesters, from 2-hydroxyheptadecan-1-yl acetate to 1-hydroxydocos-2-yl butanoate and 2-hydroxydocos-1-yl butanoate (Burger et al., 1999c), and the interdigital secretions of the bontebok and blesbok contain 12 hydroxyesters, from 2-hydroxyoctadecan-1-yl acetate to 2-hydroxydocosan-1-yl butanoate (Burger et al., 1999a). It is not clear whether these compounds have any semiochemical function because they are not very volatile.

Similarly it is not clear whether steroids such as the ubiquitous cholesterol, its derivative desmosterol, and lanosterol have a semiochemical function other than, possibly, serving as controlled-release carrier materials. Cholesterol and desmosterol have also been identified in the interdigital secretions of bontebok and blesbok (Burger et al., 1999a).

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