

# Lizard Epidermal Gland Secretions I: Chemical Characterization of the Femoral Gland Secretion of the Sungazer, *Cordylus giganteus*

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**Abstract** The giant girdled lizard or sungazer, *Cordylus giganteus*, is endemic to South Africa. It has been suggested that in this species, as in other lizard species, epidermal glands in the femoral, pre-cloacal regions, and cloacal glands are the main sources of semiochemicals and that these secretions could play an important role at different levels of the social biology of the animals. To gain a better understanding of the nature of the femoral gland secretions of the sungazer, characterization of the constituents of the secretions was carried out.

By using GC-MS analysis, in conjunction with auxiliary techniques, such as solventless sample introduction and trimethylsilyl derivatization, 53 relatively involatile compounds, including carboxylic acids, alcohols, ketones, esters, and steroids, were identified in the secretions of both sexes. The study showed that the secretions of male and female sungazers contain only semi-volatile chemicals.

**Keywords:** Femoral glands . Semiochemicals . Pheromones . Gas chromatography . Mass spectrometry . High-resolution mass spectrometry . Steroids

## Introduction

It has been suggested that semiochemical communication plays an important role in the social biology of lizards, for example, in territorial marking and social dominance (Alberts 1992; Alberts et al. 1992), self-recognition (Graves and Halpern 1991; Bull et al. 1999; Cooper et al. 1999; Labra et al. 2001), conspecific and sexual recognition (Alberts 1992; Steele and Cooper 1997; Cooper et al. 1999; Labra et al. 2001), and interspecific discrimination (Cooper and Vitt 1986).

Skin (epidermal) glands, the blood–skin barrier, cloacal glands, feces, and urine have been described as potential sources of pheromones in lizards (Mason 1992). In these animals, epidermal glands, and in particular, the femoral glands (holocrine, tubulo-follicular, sebaceous-type glands) in the femoral and pre-cloacal regions, have received the most attention (Quay 1986; Van Wyk and Mouton 1992; Dujsebayaeva 1998). Another type of epidermal gland, the “generation” gland [mostly holocrine secretory cells located in the beta-layer of the epidermis (Maderson 1967; Van Wyk and Mouton 1992)], is also known to occur in the femoral, pre-cloacal, antibrachial (fore-arm), and dorsal epidermal regions in cordylid lizards (Van Wyk and Mouton 1992).

Reports regarding the chemical nature of the material secreted by the epidermal glands of lizards are limited to studies on the femoral and pre-cloacal glands (Alberts 1990; Weldon et al. 1990; Alberts et al. 1992, 1993; Escobar et al. 2001, 2003; López and Martín 2005a, b, 2006).

Alberts (1990) reported proteins and lipids as the main components of the femoral secretion of the desert iguana, *Dipsosaurus dorsalis*, in a ratio of 4:1, respectively. The composition of the proteins varied among individuals. These proteins absorb ultraviolet light and may be used to facilitate visual detection of the deposited secretion by conspecifics (Alberts 1990; Alberts et al. 1993). In addition, volatile lipids could also act as olfactory cues for the detection of femoral secretions (Alberts 1990).

In male green iguanids, Alberts et al. (1992) found seasonal variations in the lipid composition, but in contrast to the proteins, little individual variation was observed. Volatile lipids in these secretions could have a semiochemical function (Mason and Gutzke 1990; Escobar et al. 2001, 2003). Alberts et al. (1992) identified a number of lipids, including carboxylic acids and steroid alcohols, in the femoral secretion of the lizard, *Iguana iguana*. Escobar et al. (2001, 2003) reported 49 lipids in 20 *Liolaemus* species.

As in other lizard species (Chauhan 1987; Alberts 1990; Alberts et al. 1992), these compounds belong to three main classes, alkanes, carboxylic acids, and steroids. Information on a possible pheromonal role of the epidermal gland secretions of squamates is limited. Variation in the incidence and relative amounts of specific compounds has, nevertheless, been attributed to intraspecific (Alberts 1990; Escobar et al. 2003), interpopulational (Escobar et al. 2003), and interspecific differences (Cooper and Vitt 1986; Escobar et al. 2001).

If one accepts that chemicals in the femoral secretions play a role in the communication of lizards, then this raises the question of whether the major constituents, such as the fatty acids that are almost ubiquitous in nature, are the major semiochemicals of these animals, or whether minor components, perhaps overlooked in the investigations, are involved?

To shed more light on this question, we started analyzing lizard secretions by using highly sensitive chemical methods.

The family Cordylidae (girdled lizards) is one of the lesser-known lizard families of the world and is restricted to the southern subcontinent of Africa (Frost et al. 2001). Femoral glands occur on the ventral aspect of the thigh in males, and in some species, also in the females (Mason 1992).

The cordylid femoral glands correspond to the tubulo-follicular sebaceous type holocrine glands found in other lizards (Cole 1966; Quay 1986). The so-called sungazer, *Cordylus giganteus*, a large terrestrial girdled lizard, inhabits the Highveld *Themeda* grasslands in parts of the Free State, KwaZulu-Natal, and Mpumalanga provinces of South Africa (Van Wyk 1992; Branch 1998). Both sexes possess femoral glands in the ventral femoral region of the hind legs.

Males also exhibit generation glands as a patch of glandular scales, anterior to the single row of femoral glands. In addition, males have a patch of generation glands on the ventral aspect of the antebrachial (fore-arm) region (Van Wyk and Mouton 1992). The secretions of these glands were not investigated in the present study. Due to the nature of their grassland habitat, visual communication may be of limited value, and therefore, chemical communication is a possible alternative. *C. giganteus* is also recognized as a species showing high refuge fidelity (Ruddock 2000) in a rather homogenous habitat, further suggesting the use of chemical cues for site recognition.

Here, we describe a comprehensive chemical characterization of the volatile constituents of the femoral gland secretions of *C. giganteus*. For the first time, the chemical properties of the secretions of a lizard species of the family Cordylidae are reported.

## Methods and Materials

### General

All Pyrex glassware was thoroughly cleaned with water and acetone, and heated to 500°C to remove any residual organic material. Dichloromethane (Fluka, Residue Analysis Grade) was used for extraction of organic material from collected samples. Syringes were cleaned with the same solvent.

### Collection and Sample Preparation

Femoral gland secretions were collected from individual adult male and female lizards in a study population on a farm in the Lindley district (Free State Province, South Africa; 28°01'S, 28°05'E) during the months of April 1998 (11 males and 12 females), October 1998 (5 males and 5 females), and January 1999 (5 males and 5 females).

Secretions were collected by squeezing plugs of the secretion from the femoral pores on the hind legs of the lizards, by using a pair of forceps. The collected material was transported to Stellenbosch at  $-10^{\circ}\text{C}$  and stored at  $-70^{\circ}\text{C}$  until processed for analysis. Organic constituents were extracted with 150  $\mu\text{l}$  of dichloromethane from secretions of individual lizards.

The extracts were filtered and concentrated to ca 10  $\mu\text{l}$  by slow evaporation in a nitrogen atmosphere. Samples of 0.2 to 1.0 and 1.0  $\mu\text{l}$ , of the concentrated extracts were used for qualitative and quantitative analyses, respectively. For the determination of the amount of each chemical in the femoral gland secretion, the secretion (11.44 or 10.89 mg, respectively) of a male or a female lizard was extracted with four portions of 200  $\mu\text{l}$  dichloromethane.

The extract was slowly evaporated to dryness in an inert atmosphere at  $22^{\circ}\text{C}$ , and the residual material dissolved in 20  $\mu\text{l}$  of an internal standard solution containing 49  $\mu\text{g}$  of octadecane/ml in dichloromethane. One microliter of each of these solutions was used for the analysis by GC.

## **Analytical Techniques**

Gas chromatographic (GC) analyses were performed on Carlo Erba 4160 and 5300 GCs, while the quantitative analysis was carried out with an HP 5890 Series II GC. All instruments were equipped with a flame ionization detector (FID) and split/splitless inlet systems, with the injector and detector at  $220$  and  $280^{\circ}\text{C}$ , respectively. Helium was used as carrier gas at a linear flow velocity of  $28.6\text{ cm sec}^{-1}$ . Glass capillary columns (40 m  $\times$  0.3 mm i.d.) were coated with PS-089-OH [silanol-terminated (95%)-methyl-(5%)-phenylpolysiloxane copolymer] at a film thickness of 0.25  $\mu\text{m}$ . Samples were injected in the split mode by using a split ratio of 1:10.

The volatile chemicals were thermally focused on the column at a temperature of  $30^{\circ}\text{C}$ , after which, the column was heated ballistically to  $40^{\circ}\text{C}$  and then programmed at  $8^{\circ}\text{C min}^{-1}$  from 40 to  $160^{\circ}\text{C}$ , followed by  $2^{\circ}\text{C min}^{-1}$  to  $280^{\circ}\text{C}$  (hold 60 min). Electron impact mass spectra (EI-MS) were acquired at 70 eV on a Carlo Erba QMD 1000 GC-MS instrument, using the same column and conditions as above. For the analysis of steroid derivatives, a mass range of  $m/z$  25–550 was selected. An ion source temperature of  $200^{\circ}\text{C}$  and an interface temperature of  $250^{\circ}\text{C}$  were used for all analyses. Some GC-MS analyses were carried out on a Fisons MD-800 instrument using the parameters specified above.

High-resolution GC-MS analyses of an extract from a male sungazer were performed on an AMD Intectra 604 high-resolution mass spectrometer coupled to a Carlo Erba 4160 gas chromatograph using the gas chromatographic parameters specified for the QMD 1000 instrument.

## Trimethylsilyl Derivatives

An extract of femoral gland secretion was concentrated in a Reacti-Vial left uncapped in a nitrogen atmosphere to yield approximately 1 mg of solvent-free material. Methoxylamine hydrochloride (5.23 mg, 0.0626 mmol) dissolved in pyridine (50  $\mu$ l) was added to the material.

The reaction mixture was heated in a GC oven for 15 min at 60°C, after which, the solvent was carefully blown off with nitrogen. Trimethylsilylimidazole (75  $\mu$ l, 0.511 mmol) was added to the reaction product and the reaction mixture heated at 100°C for 2 hr in a capped vial (Thenot and Horning 1972). The sample was concentrated to about 50  $\mu$ l in a nitrogen atmosphere while the reaction product was still hot. Approximately 2  $\mu$ l of this solution was used for a GC-MS analysis.

## Reference Compounds

Most of the compounds used to confirm the identity of the chemicals of the femoral gland secretions were obtained commercially or were available from previous studies. The following compounds were synthesized. *Dodecyl propenoate* (2) was synthesized by a transesterification procedure described by Rehberg (1955). Dodecanol (16.3 g, 87.5 mmol) and methyl propenoate (25.0 g, 291 mmol) were refluxed in a nitrogen atmosphere in the presence of p-toluenesulfonic acid monohydrate (2.00 g, 10.5 mmol), as catalyst, and hydroquinone (0.350 g, 3.18 mmol) as radical scavenger. After refluxing the reaction mixture for 30 min, the temperature was reduced to 62–63°C, and the methanol/methyl propenoate azeotrope formed in the transesterification reaction distilled off over a period of 4.5 hr.

The temperature was increased, and the residual methyl propenoate distilled off at 80°C, yielding dodecyl propenoate (20.654 g, 98% yield) in a purity of 97%. MS (70 eV): *m/z* (%) 168(2), 140(4), 127(9), 125(4), 113(9), 111(13), 97(23), 83(30), 73(32), 69(32), 55(100), 43(45), 41(52), 39(10), 29(28), 27(32).

*Dodecyl propanoate* (3) was likewise synthesized by the Rehberg method from dodecanol and methyl propanoate to give the target compound. MS (70 eV): *m/z* (%) 213 (1), 168(2), 140(4), 130(2), 125(3), 111(12), 97(28), 83(39), 75(73), 57(100), 55(43), 43 (36), 41(31), 39(4), 29(24), 27(5).

## Results and Discussion

GC-MS analyses of the femoral gland secretions of male and female sungazers gave total ion chromatograms (TICs) that were qualitatively identical but with some quantitative differences. A typical total ion chromatogram of an extract of the femoral gland secretion of a female sungazer is depicted in Fig. 1.

The extracts of the secretions of both male and female lizards did not contain any early eluting volatile constituents. To rule out the possibility that highly volatile material present in the secretion could have been lost when the sample was concentrated for analysis, GC-MS analysis was repeated by using solventless sample introduction (Burger et al. 1990) of the raw secretion.

Results confirmed that the secretion, as collected, was devoid of more volatile compounds commonly present in exocrine secretions and that the constituents of the secretion can be described as semi-volatile. A total of 173 chromatographic peaks were observed in the GC-FID analyses, but in the GC-MS analyses, only 53 components (Table 1) could be detected with sufficient sensitivity to yield mass spectra suitable for identification.

The quantitative GC-FID analyses showed that these 53 compounds constituted more than 90% of the total mass of the detected compounds. The components of the extract were tentatively identified by comparison of their low-resolution electron impact mass spectra with NBS, NIST, Wiley, and the in-house LECUS mass spectra libraries. Further diagnostic information on some tentatively identified steroids was obtained by interpretation of the mass spectra of their trimethylsilyl derivatives, employing published mass spectral data. The structures of most of these compounds were confirmed by gas chromatographic co-elution with authentic reference compounds.

Although a large number of homologous long-chain alkanes were identified in the pre-cloacal secretions of 20 *Liolaemus* lizard species (Escobar et al. 2001, 2003), alkanes have not been found in the secretions of other lizards. The femoral secretion of *C. giganteus* contained only one alkane, pentacosane. Constituents 1 and 7 were tentatively identified as long-chain alcohols. The molecular mass of long-chain alcohols can normally be established by invoking a characteristic ion at  $[M-46]^+$  formed by the loss of a molecule each of water and ethylene.

Although constituent 7 was present in such a low concentration that this ion could not be detected, this chemical and constituent 1 were identified by gas chromatographic co-elution with authentic synthetic reference compounds as 1-hexadecanol and 1-dodecanol, respectively. These were the only alcohols identified in the secretions. The femoral secretions of some other species that have been investigated were found to contain up to nine homologous unbranched long-chain alcohols (López and Martín 2005b; Martín and López 2006b).

A series of methyl ketones, comprising 2-heptadecanone (8), 2-nonadecanone (14), 2-henicosanone (22), 2-tricosanone (26), 2-tetracosanone (30), and 2-pentacosanone (32), was identified in the femoral secretion of the sungazer.

Previous studies have found C<sub>13</sub> and C<sub>19</sub>, C<sub>15</sub> and C<sub>16</sub>, C<sub>16</sub> and C<sub>19</sub>, and C<sub>19</sub> ketones in the femoral secretions of, respectively, *Acanthodactylus erythrurus* (López and Martín 2005b), *Lacerta schreiberi* (López and Martín 2006), *Psammodromus algirus* (Martín and López 2006a), and *Podarcis hispanica* (Martín and López 2006b). The sungazer's femoral secretion contained a single unsaturated C<sub>17</sub> aldehyde.

Martín and López (2006a, b) found only a few aldehydes in the secretions of the species they studied. Aldehydes are oxidized by oxygen to fatty acids in an autoxidation reaction that is promoted by high temperatures and ultraviolet light (Pryor 1966). It is possible that  $\alpha$ -tocopherol, a radical scavenger (Fossey et al. 1995) present in the femoral secretion of lizards (e.g., *Lacerta schreiberi*; López and Martín 2006), largely inhibits this reaction. The secretions contained 19 saturated and unsaturated C<sub>14</sub>–C<sub>24</sub> carboxylic acids. Ions characteristic of carboxylic acids are present in low abundance in the mass spectra of constituents 10, 12, 28, and 33.

Based on the available mass spectral information and retention time comparison with other saturated and unsaturated carboxylic acids, these compounds were identified as unsaturated carboxylic acids. However, the position of unsaturation in these acids was not established. Previous investigators (e.g., Alberts et al. 1992) have found carboxylic acids with similar chain lengths in other species, while still other species produce carboxylic acids with a wider range in the number of carbon atoms (e.g., C<sub>6</sub>–C<sub>26</sub> in *Liolaemus* spp; Escobar et al. 2001).

The mass spectrum of constituent 3 contained ions which are typically present in the mass spectra of long-chain alcohols and alkenes. However, comparison of this spectrum with mass spectral libraries provided no conclusive characterization: the main point of divergence being the presence (in 3) of an abundant ion at  $m/z$  75. High-resolution GC-MS analysis revealed that the elemental compositions of this ion and another at  $m/z$  57 were C<sub>3</sub>H<sub>7</sub>O<sub>2</sub> and C<sub>3</sub>H<sub>5</sub>O, respectively, indicating that constituent 3 could be an ester of propanoic acid, possibly dodecyl propanoate.

The high-resolution mass spectrum of constituent 2 showed that the prominent ions at  $m/z$  73 and 55 were C<sub>3</sub>H<sub>5</sub>O<sub>2</sub> and C<sub>3</sub>H<sub>3</sub>O, respectively. As polar compounds elute faster than analogous saturated compounds on apolar columns, it was concluded that constituent 2 is the propanoic acid ester analog of constituent 3. Gas chromatographic co-elution of these two chemicals with synthetic reference compounds confirmed that both of them contain unbranched alkyl moieties, i.e., they are dodecyl propanoate (2) and dodecyl propanoate (3).

The presence of a relatively large number of steroids, which eluted from the apolar column with retention times between approximately 87 and 105 min, in the femoral secretion of the sungazer suggested to us that these compounds could have a semiochemical function in this animal.

The interpretation of the mass spectra of steroids has been discussed in great detail (Budzikiewicz 1972; Thenot and Horning 1972; Shackleton 1985). Some of the more informative diagnostic ions in the mass spectra of the steroids identified in the femoral secretion of the sungazer and of their TMS derivatives are summarized in Table 2. Using this information and retention time comparison with authentic synthetic steroids, seven steroids were identified in the secretion.

Constituent 46 was tentatively identified as 4,4-dimethyl-cholest-8-en-3 $\beta$ -ol. However, the published mass spectral data of this steroid did not correspond well with the mass spectrum of constituent 46, and therefore, this compound remains unidentified. Another ten steroids could not be identified, some of which are also present and unidentified in other lizards (Martín and López 2006b).

The abundant steroids plus the well-known fixative squalene may function as the major controlled-release carrier materials in the secretion. Escobar et al. (2003) and Alberts (1990) postulated that cholesterol and the protein fraction could serve as controlled-release carrier materials or fixatives for semiochemicals in some lizards. It is also possible that the long-chain esters, hydroxy esters, and lactones (López and Martín 2005a, b, 2006; Martín and López 2006a, b) could have the same function in other lizard species.

Lactones, long chain esters, and hydroxy esters were not found in the femoral gland secretion of the sungazer. Squalene is the major component in the secretions collected from males during April 1998 and January 1999. In the secretions collected from males during October 1998, the component with the highest relative concentration differed among individuals and was squalene, cholest-5-en-3 $\beta$ -ol, or lanost-8-en-3 $\beta$ -ol. The major component in female secretions collected during these three seasons was usually either cholesterol or cholesta-5,7-dien-3 $\beta$ -ol, although in each of these seasons, a different female had octadecanoic acid as the major chemical.

Available information on the role of chemical communication in the social behavior of Cordylid lizard species is limited to a study of the behavior of the Cape girdled lizard, *Cordylus cordylus*, a member of the Cordilidae family, which showed that males and females can discriminate between their own secretions and those of individuals of the same sex (Cooper et al. 1999).

Further research is needed to test whether *C. giganteus* individuals respond to femoral gland secretions of conspecifics and, if so, which (if any) of the chemicals identified herein mediate such responses.



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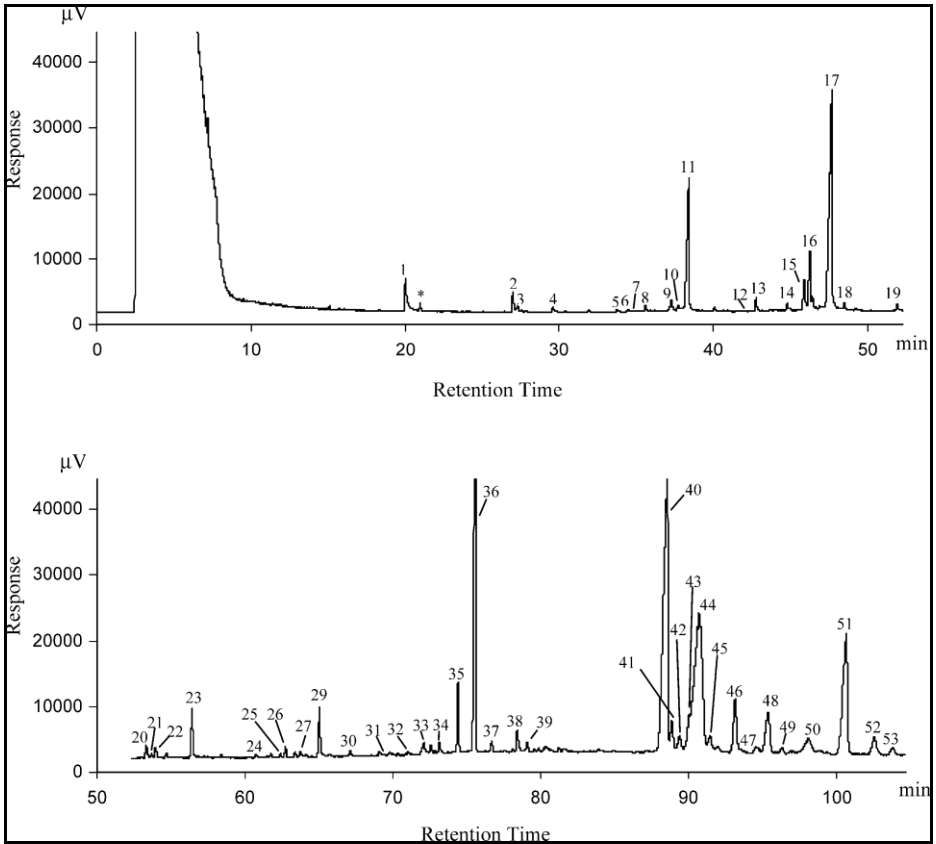


Fig. 1 Total ion chromatogram (FID) of an extract of femoral gland secretion of a female sungazer, *C. giganteus*. \*Contaminant

Table 1. Constituents identified in the femoral gland secretion of the sungazer

Number in Fig. 1 <sup>a</sup>	Constituent	Ident. <sup>b</sup>	Quantity (ng/mg) <sup>c</sup>		Male relative Amounts ± S <sup>d</sup>			Female relative Amounts ± S <sup>d</sup>		
			Male	Female	April 1998 (n=11) <sup>e</sup>	October 1998 (n=5) <sup>e</sup>	January 1999 (n=5) <sup>e</sup>	April 1998 (n=12) <sup>e</sup>	October 1998 (n=5) <sup>e</sup>	January 1999 (n=5) <sup>e</sup>
Acids										
4	Tetradecanoic acid	a,b	179	158	0.25±0.08	0.35±0.23	0.23±0.09	0.15±0.06	0.13±0.05	0.14±0.05
5	Pentadecanoic acid	a,b	135	125	0.12±0.03	0.15±0.06	0.11±0.06	0.12±0.04	0.16±0.02	0.18±0.13
9	9Z-Hexadecenoic acid	a,b	468	191	0.76±0.39	0.94±0.70	0.75±0.28	0.34±0.23	0.27±0.10	0.39±0.15
10	Unidentified (a hexadecenoic acid)	a,c,d	62	69	0.14±0.07	0.19±0.15	0.13±0.06	0.25±0.13	0.35±0.21	0.35±0.30
11	Hexadecanoic acid	a,b	4,209	2,318	7.05±2.05	8.59±3.03	7.16±2.68	4.82±1.44	4.76±1.15	4.42±2.02
12	Unidentified (a heptadecenoic acid)	a,c,d	32	21	0.03±0.03	0.03±0.01	0.02±0.02	0.05±0.06	0.02±0.01	0.02±0.01
13	Heptadecanoic acid	a,b	188	170	0.27±0.08	0.27±0.08	0.24±0.08	0.50±0.18	0.55±0.03	0.42±0.17
15	9Z,12Z-Octadecadienoic acid	a,b	394	282	1.31±0.62	1.22±0.59	1.54±0.63	1.26±0.68	1.48±0.47	1.71±0.45
16	9Z-Octadecenoic acid	a,b	1,321	1,042	1.31±0.45	1.81±0.42	1.88±0.80	2.24±1.11	2.57±0.63	3.31±1.12
17	Octadecanoic acid	a,b	2,989	3,313	6.80±1.43	6.62±1.91	6.44±2.36	10.74±3.48	12.37±3.60	10.06±5.23
19	Nonadecanoic acid	a,b	37	40	0.12±0.02	0.16±0.06	0.12±0.04	0.18±0.07	0.22±0.07	0.16±0.06
20	8Z,11Z,14Z-Icosatrienoic acid	a,c	488	270	2.27±1.58	1.49±1.08	2.64±0.79	0.69±0.46	0.29±0.11	1.07±0.39
23	Icosanoic acid	a,b	134	250	0.66±0.12	0.87±0.21	0.77±0.23	1.17±0.47	1.67±0.69	1.14±0.60
24	Henicosanoic acid	a,b	32	68	0.06±0.02	0.09±0.04	0.07±0.02	0.11±0.05	0.17±0.09	0.10±0.04
28	Unidentified (a docosenoic acid)	a,c,d	240	341	0.08±0.04	0.10±0.03	0.16±0.08	0.08±0.04	0.17±0.04	0.17±0.03
29	Docosanoic acid	a,b	38	110	0.64±0.15	0.91±0.34	0.84±0.24	1.20±0.55	1.83±0.79	1.18±0.68
31	Tricosanoic acid	a,b	44	74	0.01±0.01	0.01±0.01	0.00±0.00	0.02±0.02	0.02±0.02	0.02±0.02
33	Unidentified (a tetracosenoic acid)	a,c,d	96	85	0.13±0.04	0.20±0.19	0.20±0.13	0.17±0.11	0.18±0.13	0.20±0.13
34	Tetracosanoic acid	a,b	50	91	0.18±0.10	0.28±0.14	0.25±0.09	0.29±0.17	0.52±0.26	0.34±0.22
Alcohols										
1	1-Dodecanol	a,b	30	17	1.37±0.84	0.01±0.01	0.02±0.02	2.43±1.18	–	0.17±0.26
7	1-Hexadecanol	a,b	18	25	0.03±0.01	0.05±0.02	0.04±0.01	0.03±0.01	0.04±0.01	0.03±0.01
Ketones										
8	2-Heptadecanone	a,b	210	230	0.13±0.05	0.11±0.06	0.10±0.02	0.28±0.12	0.33±0.06	0.28±0.08
14	2-Nonadecanone	a,b	312	213	0.19±0.07	0.17±0.06	0.17±0.06	0.27±0.12	0.36±0.08	0.26±0.08

Table 1 (continued)

Number in Fig. 1 <sup>a</sup>	Constituent	Ident. <sup>b</sup>	Quantity (ng/mg) <sup>c</sup>		Male relative Amounts ± S <sup>d</sup>			Female relative Amounts ± S <sup>d</sup>		
			Male	Female	April 1998 (n = 11) <sup>c</sup>	October 1998 (n=5) <sup>c</sup>	January 1999 (n=5) <sup>c</sup>	April 1998 (n = 12) <sup>c</sup>	October 1998 (n=5) <sup>c</sup>	January 1999 (n=5) <sup>c</sup>
22	2-Henicosanone	a,b	43	42	0.22±0.06	0.22±0.06	0.23±0.06	0.44±0.16	0.49±0.10	0.39±0.08
26	2-Tricosanone	a,b	255	202	0.21±0.06	0.22±0.07	0.21±0.06	0.53±0.15	0.51±0.13	0.41±0.12
30	2-Tetracosanone	a,b	51	57	0.02±0.02	0.06±0.03	0.02±0.02	0.14±0.09	0.61±0.31	0.21±0.12
32	2-Pentacosanone	a,b	83	72	0.06±0.02	0.05±0.03	0.05±0.02	0.13±0.06	0.14±0.06	0.09±0.03
Esters										
2	Dodecyl propenoate	a,b	357	560	0.43±0.15	–	0.06±0.02	0.80±0.35	–	0.26±0.30
3	Dodecyl propanoate	a,b,g	33	63	0.09±0.03	–	0.01±0.00	0.17±0.07	0.00±0.01	0.02±0.02
Steroids										
27	Unident. ster. (217, 232, 246, 259, 302) <sup>f</sup>	a	124	123	0.01±0.01	0.06±0.04	0.02±0.02	0.13±0.07	0.32±0.18	0.26±0.18
37	Unident. ster. (135, 143, 247, 265, 366)	a	465	206	0.39±0.17	1.12±0.80	0.75±0.51	0.33±0.14	0.60±0.22	0.57±0.18
38	Unident. ster. (155, 197, 251, 349, 364) <sup>g</sup>	a	371	506	0.44±0.27	0.55±0.25	0.32±0.14	0.93±0.18	1.17±0.31	0.89±0.43
39	Unident. ster. (195, 209, 249, 349, 364)	a	140	132	0.07±0.05	0.08±0.02	0.07±0.02	0.29±0.07	0.33±0.07	0.26±0.09
40	Cholest-5-en-3β-ol	a,b,e	7,279	7,155	10.44±2.44	15.94±3.19	10.10±2.37	15.46±3.43	18.74±5.63	15.57±6.97
41	Unident. ster. (217, 233, 351, 384, 388, 441, 456)	a	626	656	0.47±0.10	0.63±0.11	0.56±0.11	0.96±0.36	0.98±0.34	1.21±0.38
42	Unident. ster. (213, 229, 273, 353, 371, 386)	a	1,709	217	0.07±0.03	0.10±0.07	0.07±0.02	0.34±0.15	0.75±0.27	0.22±0.08
44	Cholesta-5,7-dien-3β-ol	a,b,e	6,783	3,703	5.44±2.07	4.06±1.76	4.78±2.64	16.07±4.64	11.54±5.49	15.11±4.64
45	Unident. ster. (213, 237, 335, 350, 368)	a	69	144	0.13±0.08	0.10±0.08	0.11±0.06	0.40±0.16	0.45±0.15	0.28±0.20
46	Unident. ster. (259, 273, 340, 381, 399, 414)	a,e	2,066	1,080	2.22±0.87	3.45±1.39	2.59±0.87	1.84±0.86	2.20±0.26	2.56±0.98

47	Unident. ster. (231, 243, 257, 275, 397, 412)	a	137	295	0.27±0.06	0.45±0.24	0.34±0.13	0.38±0.18	1.09±0.92	0.56±0.40
48	Ergost-5-en-3β-ol	a,e	1,048	946	1.18±0.31	1.96±0.48	1.40±0.25	1.96±0.49	2.35±0.46	2.10±0.97
49	Cholest-4-en-3-one	a	110	666	0.07±0.06	0.26±0.20	0.20±0.33	0.23±0.32	2.82±4.48	1.45±3.00
50	Unident. ster. (257, 271, 339, 365, 398, 411, 426)	a	901	878	1.04±0.29	1.24±0.58	1.03±0.44	1.49±0.54	3.38±2.57	1.84±0.74
51	Lanost-8-en-3β-ol	a,e	7,761	4,212	15.13±4.20	18.32±4.30	14.57±2.55	8.60±3.28	7.68±1.98	9.00±3.29
52	Stigmast-5-en-3β-ol	a,e	368	236	0.51±0.24	0.77±0.26	0.59±0.17	0.79±0.35	1.27±0.38	1.00±0.35
53	Lanosterol	a,b,e	532	360	0.72±0.15	1.46±0.77	0.94±0.16	0.48±0.28	0.62±0.25	0.61±0.20
Other										
6	2-Heptadecenal	a,c,f	209	67	0.11±0.06	0.21±0.12	0.13±0.03	0.07±0.04	0.11±0.03	0.06±0.04
21	Unidentified alkene	a	28	15	0.05±0.05	0.06±0.07	0.06±0.03	0.08±0.06	0.02±0.01	0.04±0.03
25	Pentacosane	a,b	62	63	0.01±0.01	0.02±0.02	0.02±0.01	0.13±0.09	0.03±0.03	0.05±0.02
36	Squalene	a,b	1,535 <sup>9</sup>	2,376	30.50±4.86	17.76±8.10	31.42±5.58	8.57±2.28	3.67±1.22	9.33±2.85
43	α-Tocopherol	a,b	606	323	1.71±0.83	0.99±0.53	1.82±0.99	2.14±1.54	1.61±1.00	2.64±1.21
18	Unidentified (185, 222, 264, 284, 296)	a	131	169	0.01±0.01	0.02±0.01	0.01±0.01	0.06±0.04	0.06±0.04	0.07±0.04
35	Unidentified (111, 125, 153, 243, 253, 282)	a	80	155	0.23±0.20	0.34±0.14	0.16±0.07	1.15±1.62	0.62±0.40	0.65±0.55

a GC-MS analysis, b retention time comparison with synthetic compounds, c retention time increment comparison, d position of double bond unknown, e EI-spectra of TMS derivatives, f E/Z-isomerism unknown, g GC-HRMS

<sup>a</sup> In order of elution from GC column

<sup>b</sup> Identification

<sup>c</sup> Mass of each chemical in 1 mg of secretion

<sup>d</sup> Relative amounts are calculated as a percentage of the total 173 compounds detected by GC-FID. The mean ± SD is reported.

<sup>e</sup> The same five lizards were sampled in each of the three seasons or were present in the larger samples

<sup>f</sup> Prominent ions in the mass spectra of unidentified steroids

<sup>g</sup> Ions corresponding to those in the mass spectrum of an unidentified steroid found in the femoral gland secretion of *Podarcis hispanica* (Martín and López 2006b).

Table 2 Diagnostic ions and normalized abundances<sup>a</sup> from the mass spectra of the TMS derivatives of the steroids identified in the femoral gland secretion of the sungazer

TMS-derivative of:	M	M-15 <sup>b</sup>	M-90 <sup>c</sup>	M-(90+15)	M-129 <sup>d</sup>	M-(sc. <sup>e</sup> + 90)	M-(D-ring <sup>f</sup> + 90)	M-(C-ring <sup>g</sup> + 90)
Cholest-5-en-3 $\beta$ -ol	458	443	368	353	329	255	213	145
	<i>7<sup>a</sup></i>	2	26	15	32	14	10	33
Cholesta-5,7-dien-3 $\beta$ -ol	456	441	366	351	<i>a</i>	253	211	143
	5	$\geq 0$	16	70	–	18	25	64
Ergost-5-en-3 $\beta$ -ol	472	457	382	367	343	255	213	145
	4	2	24	10	24	9	9	28
Lanost-8-en-3 $\beta$ -ol	500	485	410	395	–	297	255	173/175
	5	10	2	100	–	2	9	20/19
Stigmast-5-en-3 $\beta$ -ol	486	471	396	381	357	255	213	145
	5	2	17	8	18	11	10	34
Lanosterol	498	483	(408) <sup>h</sup>	393	–	297	255	173/175
	5	9	–	38	–	3	14	13/9

<sup>a</sup> M-131 is however formed at m/z 325, with a relative abundance of 62%

<sup>a</sup> Normalized abundances are given in italics

<sup>b</sup> Loss of a methyl group

<sup>c</sup> Loss of TMSOH

<sup>d</sup> Loss of TMSO(CH)<sub>2</sub>CH<sub>2</sub>

<sup>e</sup> sc. = side chain on C17

<sup>f</sup> Cleavage of the D-ring

<sup>g</sup> Cleavage of the C-ring

<sup>h</sup> Does not appear in the mass spectrum, due to the low concentration of the compound

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