Chemical signals in desert lizards: are femoral gland secretions of male and female spiny-tailed lizards, *Uromastyx aegyptia microlepis* adapted to arid conditions?

José Martín[a], Aurora M. Castilla[b,c], Pilar López[a], Mohammed Al-Jaidah[d], Salman F. Al-Mohannadi[e], Ahmad Amer M. Al-Hemaidi[b]

[a]*Departamento de Ecología Evolutiva, Museo Nacional de Ciencias Naturales, CSIC. José Gutiérrez Abascal 2, 28006 Madrid, Spain. E-mail* : Jose.Martin@mncn.csic.es
[b]*Ministry of Environment, P.O. Box 7634, Doha, Qatar.*
[c]*Qatar Environment and Energy Research Institute (Hamad Bin Khalifa University), Qatar Foundation, P.O. Box 5825, Doha, Qatar.*
[d]*Environment Department, Qatar Armed Forces, Ministry of Defence, Qatar.*
[e]*Industrial Cities Directorate, Qatar Petroleum, Qatar.*

ABSTRACT

Many lizards use femoral gland secretions in intraspecific chemical communication, but specific compounds have been identified in only a few species. Chemical composition of secretions may depend on phylogeny, but it may also evolve to maximize efficacy of signals in a given environment. In deserts, the extreme dry and hot environmental conditions are hostile for chemical signals and, therefore, we expected desert lizards to have secretions with highly stable compounds. Using GC-MS, we identified 74 lipophilic compounds in femoral secretions of male and female spiny-tailed lizards, *Uromastyx aegyptia microlepis* (Fam. Agamidae), from the Qatar desert. Compounds included mainly steroids and fatty acids, but also terpenoids, ketones, tocopherol, aldehydes and alcohols. We found differences between males and females; males had higher proportions of fatty acids and tocopherol, but lower proportions of ketones than females. Contrary to expectations, the most abundant compounds were not stable in the desert climatic conditions at the surface. However, secretions could be rather adapted to microclimatic conditions inside burrows where these lizards spend long periods of time. We suggest that in addition to phylogenetic and environmental characteristics, we should know the ecology of a lizard species before making generalizations on the potential characteristics of its chemical signals.

Keywords: Reptiles, Lizards, *Uromastyx*, Femoral secretions, Intraspecific communication, Qatar desert

1. Introduction

In many lizard species, males have conspicuous femoral or preanal glands that secrete chemical compounds (Weldon et al., 2008), which are important in intraspecific communication (Mason, 1992; Mason and Parker, 2010; Martín and López, 2011; Wyatt, 2014). The proportions of some compounds allocated to secretions often depend on a male's condition (Martín and López, 2015), which allows honest signaling of the size, age or health state (e.g. López et al., 2006; Martín et al., 2007a; López and Martín, 2012; Heathcote et al. 2014). Because this information is reliable, it may be used by females to choose potential mates (e.g., Martín and López, 2000, 2006a,b, 2012; Kopena et al., 2011; López et al., 2002, 2003; Olsson et al., 2003; López and Martín, 2005a), or by other males to assess fighting ability or dominance status of competitor males (Aragón et al., 2001; López and Martín, 2002, 2011; Carazo et al., 2007; Martín and López, 2007; Martín et al., 2007b; Khannoon et al., 2011b).

Female lizards also often possess femoral pores, but in most species they are much smaller and with very little or without apparent secretion (Mason, 1992). Female femoral secretions have been overlooked in the literature, probably because they are not as prominent as in males, secretions of females are difficult to collect and analyze. However, femoral secretions do appear in females of some species, as in the lacertid lizard *Acanthodactylus boskianus*, where the same compounds, although in different proportions, are found in both sexes (Khannoon et al., 2011a). Also, female sagebrush lizards, *Sceloporus graciosus*, have active femoral glands during the mating season that produce secretions with unknown compounds (Kelso and Martins, 2008). In the desert iguana, *Dipsosaurus dorsalis*, females also produce secretion but only when they are unmated, suggesting that this secretion may be used to attract potential mates (Alberts, 1990). Secretion of female lizards may be more common than previously though and, therefore, it is worth to examine the existence of femoral secretions in females of other lizard species too and analyze these secretions.

With respect to the type of compounds found in femoral gland secretions of lizards, both lipids and proteins are found, but lipophilic compounds seem more important in intraspecific communication in most species (Mason, 1992; Martín and López, 2006a, 2011). The presence and abundance of specific compounds seem to vary consistently and widely among species (Weldon et al., 2008). It is not clear whether this interspecific variation is explained more by phylogenetic than by environmental differences (Alberts,

1992; Escobar et al., 2003; Martín and López, 2006c, 2013). With respect to phylogeny, however, most information on chemical composition of secretions relates to lizard species within the Scleroglossa clade (Weldon et al., 2008), which are considered to rely more on chemical senses (Cooper, 1995). Within this clade, specific compounds in secretions are known for males of a few species, including mainly Mediterranean lacertids (e.g. López and Martín, 2005b, c, 2006, 2009; Martín and López, 2006c,d, 2010a; Gabirot el al., 2008, 2010; Kopena et al., 2009; Khannoon et al., 2011a,b; Martín et al., 2013c), one cordylid (Louw et al., 2007), one teid (Martín et al., 2011) and a few gekkonids (Khannoon, 2012).

In contrast, compounds in secretions of lizards within the Iguania clade have been less studied, probably because these lizards were thought to rely more on visual cues (Cooper, 1995). However, lizard species from many Iguanian families have femoral or preanal glands and are capable of chemosensory conspecific recognition (Mason and Parker, 2010). Within this clade, lipophilic compounds in gland secretions have been described only in males of two iguanid species (Weldon et al., 1990; Alberts et al., 1992a,b; Martín et al., 2013a) and of several south American tropidurid species within the genus *Liolaemus* (Escobar et al., 2001, 2003). Information on chemical signals of agamids is also very scarce. An old study using thin layer chromatography (TLC) analyzed compounds in preanal secretions of male *Uromastyx hardwickii* lizards, suggesting the presence of fatty acids, triacylglycerols, wax esters, sterols and their esters, and phospholipids (Chauhan, 1986). In addition, a recent study examined femoral secretions of males of the African tree agama (*Acanthocercus atricollis*), finding as main compounds steroids (mainly cholesterol and cholest-3-ene), fatty acids, and a series of saturated methyl ketones (Martín et al., 2013b). To understand the phylogenetic influence on composition of femoral gland secretions of lizards, we need more studies that deal with a wider range of lizard species within different taxonomic groups. In this context, more studies of agamid lizards are required.

With respect to the environmental influences, we need studies that consider species inhabiting a larger variety of environmental conditions. This is because signals used in intraspecific communication are expected to evolve to maximize efficacy of the signal in a given environment (Guilford and Dawkins, 1991; Endler and Basolo, 1998; Bradbury and Vehrencamp, 2011). Factors such as how the signal transmits through the environment, durability or persistence of the signal, or how well the signal is detected by the receivers, are selective factors modeling the efficacy design of the sexual signals (Guilford and Dawkins, 1991; Alberts, 1992; Endler and Basolo, 1998; Boughman, 2002; Martín and

López, 2013). All these variables largely depend on the climatic conditions. For example, within Iberian *Podarcis* lacertid lizards, the relative proportion of cholesterol in femoral secretions increases in populations inhabiting more hot and dry habitats (Gabirot et al., 2012), presumably because cholesterol would confer more stability to secretions giving them greater longevity (Alberts, 1992; Escobar et al., 2003). Deserts are environments especially hostile for chemical signals because dryness and high temperatures can strongly and quickly alter the properties of compounds (Martín and López, 2013) and, thus, destroy the potential messages of the signal. Therefore, we would expect to find higher abundances of the more stable compounds in femoral secretions of lizards living in these extreme dry and hot habitats.

In the present paper, we report the results of an analysis by gas chromatography–mass spectrometry (GC-MS) of the lipophilic fraction of femoral gland secretions of male and female spiny-tailed lizards, *Uromastyx aegyptia microlepis* (Agamidae), from the Qatar desert (Castilla et al., 2014; Cogălniceanu et al., 2014). This is a large (mean male body mass of 1,600 g), heliothermic and actively foraging mainly herbivorous agamid lizard (Cunningham, 2000; Al-Hazmi, 2001; Wilms et al., 2010; Castilla et al., 2011; Herrel et al., 2014) that inhabits deserts and semi-deserts of North Africa and the Middle East (Naldo et al., 2009; Cogălniceanu et al., 2014). Both males and females have femoral pores with active secretions during the mating season. Because of the large size of this lizard, the femoral secretions of females could be easily collected and analyzed too. We specifically a) described the composition and relative proportions of compounds in femoral secretions, b) examined whether secretions of males and females were different, and c) compared the compounds in secretions with those of other agamid lizards. In addition, we discuss whether these secretions could be useful in the desert climatic conditions given the chemophysical properties of the compounds found.

2. Material and methods

2.1. Species and study area

During spring-summer 2012, we captured *U. aegyptia* lizards, 29 males (snout-to-vent length, SVL: mean $\pm$ SE = 256 $\pm$ 10 mm) and 15 females (SVL: 237 $\pm$ 15 mm), in the Qatar desert, an area characterized by high aridity (average annual mean temperature of 29 °C; average annual rainfall of 45 mm). The habitat where the lizards were captured was an open rocky and sandy area with scattered bushes (e.g., *Lycium shawii*, *Tetraena qatarensis*,

Fagonia indica, Suaeda sp.), acacia trees (*Acacia tortilis*) and several perennial herbs. Lizards were captured alive by hand and taken to the laboratory in QEERI (Qatar Foundation) where measurements of the animals and secretion collection was made. Lizards were returned to the capture sites after measurements.

2.2. Chemical analyses

We extracted secretion from femoral glands of live lizards by pressing around the femoral pores. Secretion was directly collected with glass inserts that were kept in glass vials closed with Teflon-lined stoppers. Vials were stored at -20 °C until analyses. We prepared some blank control vials using the same procedure, but without collecting secretion, which could be compared with the lizards samples to exclude contaminants from the handling procedure and for examining potential impurities in the solvent or laboratory instruments.

We analysed samples with a ThermoQuest (Austin, TX, USA) Trace 2000 gas chromatograph fitted with a poly(5 % diphenyl/ 95 % dimethylsiloxane) column (Supelco, Equity-5, 30 m length x 0.25 mm ID, 0.25 μm film thickness, from Supelco Co., Bellefonte, PA, USA) and a ThermoQuest Trace 2000 mass spectrometer as detector. We injected in splitless mode 2 μl of each sample previously dissolved in 2 ml of *n*-hexane (capillary GC grade, from Sigma-Aldrich Chemical Co. St. Louis, MO, USA). Helium was the carrier gas. Injector and detector temperatures were set at 250 °C and 280 °C, respectively. The oven temperature program started at 50 °C isothermal for 3 min, then it was increased to 300 °C at a rate of 5 °C/min, and finally maintained isothermal at 300 °C during 15 min. Mass spectral fragments below $m/z = 46$ were not recorded. Impurities identified in the control vial samples are not reported. To perform an initial identification of secretion components, we compared mass spectra of compounds in the sample with those in the NIST/EPA/NIH (NIST 02) computerized mass spectral library. Identifications were confirmed by comparing spectra and retention times with those of authentic standards when they were available (from Sigma-Aldrich Chemical Co. St. Louis, MO, USA).

2.3. Statistical analyses

We determined the relative amount of each compound in the sample as the percent of the total ion current (TIC). To correct the problem of nonindependence of proportions,

we first logit transformed the proportion data by taking the natural logarithm of proportion ∕ (1 – proportion) (Aebischer et al., 1993). We calculated Euclidean distances between every pair of individual samples to produce a resemblance matrix that was the basis of further analyses. To analyze whether the chemical profiles of the femoral secretions varied between males and females, we used a single factor permutational multivariate analysis of variance test (PERMANOVA, Anderson, 2001; McArdle and Anderson, 2001) based on the Euclidean resemblance matrix using 999 permutations. We also used canonical analysis of principal coordinates (CAP, Anderson and Willis, 2003) to test for differences between sexes. These statistical tests were made with the software PRIMER V6.1.13 (Clarke and Gorley, 2006) with the PERMANOVA+ V1.0.3 add-on package (Anderson et al., 2008).

3. Results

Male *U. aegyptia microlepis* had a significantly higher number of femoral pores than females (males: 17.7 $\pm$ 0.4 pores/side, range = 13-20; females: 15.2 $\pm$ 1.1 pores/side, range = 11-20; $F_{1,42} = 7.06$, $P = 0.011$). Both males and females had active secretion from these pores, although secretion was visibly more abundant in males.

Overall, a total of 74 lipophilic compounds were identified in the femoral gland secretions of males and females (Table 1). Males and females shared 52 compounds (70.3 % of total of compounds), which comprised 97.9 % of the overall TIC area. However, there were some differences between sexes. Males had 65 compounds of which 13 were exclusive compounds of males (20.0 % of the compounds found in males, but only 2.9 % of TIC area of males), whereas females had 61 compounds of which only 9 were only found in females (14.7 % of the compounds of females, 1.4 % of TIC area).

In males, the main components were 42 steroids (75.5 % of TIC), 11 carboxylic acids and their esters ranged between n-C_{10} and n-C_{18} (7.3 %), two terpenoids (5.3 %), four ketones ranged between n-C_{17} and n-C_{22} (4.5 %), tocopherol (4.3 %), two alcohols (2.8 %) and three aldehydes (0.3 %) (Table 1). On average, the six most abundant compounds in males were cholesta-2,4-diene (10.6 % of TIC), cholestan-3-one (9.9 %), cholesterol (9.3 %), 4,22-stigmastadiene-3-one (8.7 %), cholesta-3,5-diene (7.5 %) and squalene (4.9 %). Major compounds were detected in all individuals, although relative proportions of some compounds showed interindividual variability.

In females, the main components were 45 steroids (80.4 % of TIC), five ketones ranged between n-C_{17} and n-C_{30} (6.7 %), two terpenoids (4.5 %), seven carboxylic acids

and their esters between n-C_{14} and n-C_{18} (2.5 %), one alcohol (3.7 %) and tocopherol (2.2 %) (Table 1). On average, the five most abundant compounds in females were cholestan-3-one (10.7 % of TIC), cholesta-2,4-diene (9.9 %), cholesterol (9.4 %), cholesta-3,5-diene (7.8 %) and 4,22-stigmastadiene-3-one (7.3 %).

The PERMANOVA based on the resemblance matrix comparing the chemical profiles of femoral secretions of males and females showed statistically significant overall differences between sexes (pseudo $F_{1,42}$=4.25, $P = 0.045$). The CAP analysis classified on average 97.7 % of the chemical profiles into the correct sex ($\delta_1^2 = 0.99$, $P = 0.001$, using leave-one-out cross-validation and $m = 18$ axes). The degree of correct classification was 100 % for males and 93.3 % for females.

Comparing between sexes the relative abundance of the different types of compounds, males had significantly relatively higher proportions of carboxylic acids ($F_{1,42} = 5.65$, $P = 0.022$) and tocopherol ($F_{1,42} = 7.08$, $P = 0.011$) and lower proportions of ketones ($F_{1,42} = 4.59$, $P = 0.038$) than females. However, proportions of steroids ($F_{1,42} = 1.13$, $P = 0.29$), terpenoids ($F_{1,42} = 0.56$, $P = 0.46$) and alcohols ($F_{1,42} = 0.58$, $P = 0.45$) did not significantly differ between sexes. Aldehydes were only found in 20.7 % of individual males, and this may explain why males did not significantly differ ($F_{1,42} = 1.52$, $P = 0.22$) from females where we did not find any aldehyd.

4. Discussion

The lipophilic fraction of femoral secretions of *U. aegyptia microlepis* lizards is formed mainly by steroids, with minor contributions of fatty acids, terpenoids, ketones, tocopherol and other compounds. This composition is similar, but not identical, to what has been found in other agamid lizards (Chauhan, 1986; Martín et al., 2013b). Unfortunately, the only study made with lizards of the same genus *Uromastyx*, used very different analytical techniques (i.e. thin layer chromatography) (Chauhan, 1986), so our results are hardly comparable with these previous data. Our study also reveals that, although both sexes shared most compounds, there are significant intersexual differences in presence and relative proportions of compounds in secretions of *U. aegyptia microlepis*.

With respect to the steroids, there was not a predominant compound forming the bulk of secretions of *U. aegyptia*, as it occurs in many lacertid lizards, where cholesterol may account for more than 50% of TIC area in many species (Weldon et al., 2008). In contrast, in *U. aegyptia* there were five or six major steroids with more or less similar

proportions ranging between 7-10 % of the TIC area each. This is, however, similar to what occurs in secretions of the tree agama (Martín et al., 2013b). Moreover, cholesta-2,4-diene and cholestan-3-one, were the two most abundant steroids of *U. aegyptia*, with cholesterol being only the third one. Nevertheless, most of the identified steroids of *U. aegyptia* were previously found in secretions of other lizard species (Weldon et al., 2008), although there is a large variety of derivatives and unidentified (probably unknown) minor steroids that could be exclusive of this species.

Among steroids found in *U. aegyptia microlepis* and that have not been found in other lizards, it is noterworthy the presence of desmosterol, a sterol intermediate in cholesterol synthesis generated during the metabolic pathway that transforms lanosterol into cholesterol. In mammals, steroid hormones, such as progesterone or pregnenolone, may inhibit cholesterol synthesis at the step between lanosterol and cholesterol, leading to the accumulation of desmosterol in tissues (Lindenthal et al., 2001). Therefore, we could speculate that the relationship between proportions of cholesterol and desmosterol in secretions of *U. aegyptia* might be related to hormone levels, which might have a secondary signaling function of the endocrine or reproductive state of an individual (Martín and López, 2015), provided that lizards were able to detect proportions of this compound with chemosensory cues. This prompts to further specific studies examining relationships between hormone levels, the steroids found in secretions, and their potential signaling role.

4.1. Intersexual differences

Although female *U. aegyptia microlepis* have a lower number of femoral pores than males, females also have active secretion from these pores, although in a lower amount in comparison to males. Compounds were similar in both sexes, with most of compounds, including the major ones, shared by males and females. However, we found significant differences in chemical profiles between sexes mainly related to the relative proportions of some compounds. Similarly, in the lacertid lizard *Acanthodactylus boskianus*, the same compounds are found in males and females, although there are some small differences in proportions of compounds (Khannoon et al., 2011a). Intersexual differences in femoral secretions of *U. aegyptia microlepis* allowed predicting statistically the sex of an individual based on its chemical profile, and, thus, it is likely that lizards are also able to easily discriminate, by chemosensory cues, the sex of conspecifics. Although this

possibility has not yet been experimentally tested in this species, it may be expected, considering the chemosensory responses of this lizard to prey chemicals (Cooper and Al-Johany, 2002) and that most lizard species have well known sex discriminatory abilities (Mason and Parker, 2010; Martín and López, 2011) .

Among the differences observed between sexes, the lower proportions of fatty acids and tocopherol in female secretions could indicate a different function of femoral secretions in males and females that merits further studies. If it is costly to allocate dietary acquired compounds, such as oleic acid or tocopherol to secretions, deviating them from their important metabolic functions, males may use secretion of these compounds to signal their condition-dependent quality (Martín and López, 2015). Similarly, in some lacertid lizards, males that allocate more oleic acid (Martín and López, 2010b) or more tocopherol (Kopena et al., 2011) to secretions, are those in a better health condition and/or with a diet of higher quality, and their scents are more attractive to females. In contrast, females would not need to signal their quality to males in this way and could avoid secreting these costly compounds. Nevertheless, the exclusive presence in female secretions of some steroids of vegetal origin, such as some lupanols and oleanes, might be also indicators of the diet quality and breeding potential of a female.

Interestingly, femoral secretions of *U. aegyptia microlepis* have a series of saturated methyl ketones with mostly odd-numbered carbon chains, which are relatively more abundant in females. A similar bishomologous series of C_{17}–C_{25} methyl ketones were found in the femoral gland secretions of the tree agama, *A. atricollis* (Martín et al., 2013b), but also in secretions of the phylogenetically unrelated South African sungazer, *Cordylus giganteus* (Cordylidae) (Louw et al., 2007) and in the skin of female geckos *Eublepharis macularius* (Mason and Gutzke, 1990) and female *Thamnophis sirtalis* snakes (Mason et al., 1990). These methyl ketones are produced by β-oxidation of free fatty acids followed by decarboxylation (Ahern and Downing, 1974), which might explain the relative low proportions of fatty acids in female *U. aegyptia*. Similar series of ketones, but produced by skin glands, have a main role in the social and sexual behavior of red garter snakes (Mason et al., 1990), so it would be interesting to test whether ketones might have a similar role in these lizards.

4.2. Are secretions adapted to desert climatic conditions?

With respect to the environmental influences, contrary to our expectations for a lizard species living in an extremely hot and dry desert environment, the more abundant steroids, but cholesterol, are not especially stable compounds that could protect other semiochemicals in secretions in the desert climatic conditions. In contrast, the most abundant steroids in secretions, such as cholesta-2,4-diene and cholestan-3-one are relatively unstable and volatile steroids that would disappear very quickly under hot temperatures (Lide and Milne, 1993). Similarly, with respect to fatty acids, we might expect in areas with higher temperatures the presence of fatty acids of high molecular weight, and, therefore, less volatile (Alberts, 1992), which does not match with the medium chain lengths (between C_{14} and C_{18}) of the most abundant fatty acids of *U. aegyptia.* These mismatches might initially suggest that chemical signals of this lizard have not evolve to maximize efficacy of the signal in the desert environment, as it could be expected for a sexual signal (Guilford and Dawkins, 1991; Alberts, 1992; Endler and Basolo, 1998; Bradbury and Vehrencamp, 2011).

This apparent contradiction between climatic conditions and physical properties of chemical signals could, however, be explained attending to the ecology and behavior of this lizard. Thus, secretions could not be useful in the desert surface as scent marks, but they could be rather adapted to the microclimatic conditions inside burrows where these lizards spend long periods of time (Bouskila, 1983; Wilms et al., 2010). These burrows retain temperature as well as humidity very efficiently; temperatures within burrows are relatively constant, within the thermal range of the species, providing shelter from the unfavorable thermal conditions in the surrounding habitat (Wilms et al., 2010). In fact, the relatively high proportions in secretions of *U. aegyptia* lizards of tocopherol and squalene, two antioxidants, might protect secretions from the relatively higher humidity that scent marks could experience inside burrows in comparison with the extreme dry conditions outside. Femoral secretions could be used to scent-mark these burrows, probably signaling the identity of the owner and its characteristics to either competitor males or prospective mates. Similarly, *Tiliqua* skinks, which are mainly active around the entrance of their burrows, scent mark these burrows with scats, and use information in these chemical signals to decide burrow use (Fenner and Bull, 2011). We suggest that we should know the ecology and social behavior of a lizard species before making generalizations on the

potential characteristics of its chemical signals based just on its phylogenetic position and the environmental characteristics in its habitat.

Acknowledgements

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Table 1

Lipophilic compounds found in femoral gland secretions of adult male and female spiny-tailed lizards, dhub, *Uromastyx aegyptia microlepis*, from the Qatar desert. The relative content of each component was determined as the percent of the total ion current (TIC) and is reported as the average (± 1SE). Characteristic ions (*m/z*) are reported for some unidentified compounds. RT, retention time

RT	Compound	Males (*n* = 29)			Females (*n* = 15)		
	Aldehydes:						
9.8	Nonanal	0.14	±	0.14	-		
14.2	Decanal	0.16	±	0.16	-		
20.4	Dodecanal	0.04	±	0.04	-		
	Carboxylic acids:						
17.5	Decanoic acid	0.06	±	0.05	-		
24.8	Dodecanoic acid	0.01	±	0.01	-		
29.0	Tetradecanoic acid	0.25	±	0.13	0.05	±	0.05
29.5	Tetradecanoic acid, 1-methylethyl ester	0.21	±	0.11	0.46	±	0.15
30.9	Pentadecanoic acid	0.13	±	0.13	-		
31.8	14-Methyl-pentadecanoic acid, methyl ester	0.02	±	0.01	-		
32.5	Hexadecenoic acid	0.15	±	0.08	0.02	±	0.02
32.8	Hexadecanoic acid	2.12	±	0.66	0.50	±	0.18
33.5	Hexadecanoic acid, 1-methylethyl ester	0.93	±	0.46	0.91	±	0.73
35.4	14-Methyl-hexadecanoic acid, methyl ester	-			0.01	±	0.01
36.0	9-Octadecenoic acid	1.71	±	0.69	0.61	-	0.18
36.5	Octadecanoic acid	1.72	±	0.93	-		
	Alcohols:						
22.2	Dodecanol	0.15	±	0.15	-		
36.6	Octadecanol	2.67	±	1.44	3.69	±	2.01
	Ketones:						
31.3	2-Heptadecanone	0.44	±	0.08	1.13	±	0.29

35.0	2-Nonadecanone	1.11	±	0.28	2.03	±	0.44
38.4	2-Heneicosanone	1.00	±	0.31	1.62	±	0.42
41.4	2-Docosanone	1.90	±	1.72	1.91	±	1.80
49.2	12-Tricosanone		-		0.05	±	0.05
	Terpenoids:						
45.8	Squalene	4.88	±	2.55	4.36	±	1.15
47.4	Unidentified terpenoid?	0.42	±	0.17	0.16	±	0.09
	Steroids:						
46.4	Cholesta-2,4-diene	10.57	±	1.56	9.88	±	2.75
46.5	Cholest-2-ene	1.43	±	1.08	1.05	±	0.33
46.6	Cholest-3-ene	0.17	±	0.05	0.35	±	0.12
46.9	Coprostan-3,5,24-trien	1.54	±	0.54	1.14	±	0.46
47.0	Cholesta-4,6-dien-3-ol	1.50	±	0.58	2.34	±	0.75
47.3	Cholesta-3,5-diene	7.49	±	0.96	7.78	±	0.97
47.7	Coprostan-3,5,24-trien, derivative?	0.90	±	0.26	0.79	±	0.16
47.9	Unidentified Steroid (213,228,253,326,352,367)	0.35	±	0.17	0.36	±	0.27
48.0	Unidentified Steroid (197,251,347,362,376,395)	1.27	±	0.49	2.68	±	0.91
48.1	Unidentified Steroid (161,261,368,383,400,415)	0.01	±	0.01	0.08	±	0.08
48.7	Unidentified Steroid (187,213,242,281,382,407)	0.06	±	0.06	0.05	±	0.05
49.0	Unidentified Steroid (145,187,281,273,392,418)		-		0.02	±	0.02
49.2	3-Methoxy-cholest-5-ene	3.42	±	1.07	3.72	±	0.78
49.3	Unidentified Steroid (254,281,352,367,385,401)	0.79	±	0.39	0.58	±	0.38
49.4	3-Methoxy cholestane	0.02	±	0.02	0.15	±	0.08
49.5	Unidentified Steroid (215,341,356,395,402,417)	0.08	±	0.03	0.07	±	0.02
49.7	3-Methoxy-cholest-7-en-6-ol?	0.34	±	0.33		-	
49.9	Unidentified Steroid (251,287,315,353,385,392)	0.62	±	0.48	0.24	±	0.09
50.3	Cholesterol	9.29	±	0.82	9.43	±	2.75
50.4	Cholestan-3-ol	0.52	±	0.38	0.20	±	0.20
50.8	Cholestan-3-one	9.87	±	2.26	10.75	±	2.40
51.1	4,22-Stigmastadiene-3-one	8.73	±	2.90	7.30	±	1.79
51.4	Cholesta-5,24-dien-3-ol (=Desmosterol)	1.79	±	0.24	1.37	±	0.31

51.5	Ergost-22-en-3-ol	0.46	±	0.11	1.74	±	0.41
51.6	Unidentified Steroid (147,354,369,385,402,430)	0.04	±	0.03		-	
51.7	Unidentified Steroid (215,258,392,410,425)	0.12	±	0.09	0.15	±	0.11
51.8	Cholest-4-en-3-one	3.23	±	1.20	1.74	±	0.70
52.2	Cholestan-3-one, methyl derivative?	0.65	±	0.15	0.38	±	0.10
52.3	4,22-Cholestadien-3-one	1.70	±	0.38	0.70	±	0.31
52.4	Unidentified Steroid (244,287,313,340,382,410)	1.13	±	0.29	0.88	±	0.14
52.8	Lanosta-8,24-dien-3-ol	0.82	±	0.29	1.05	±	0.98
52.9	Lanost-8-en-3-ol	1.59	±	1.03	0.04	±	0.04
53.0	Cholestan-3-one, ethyl derivative?	1.55	±	0.50	3.11	±	0.70
53.1	Cholest-5-en-3-one	0.02	±	0.02	0.30	±	0.30
53.2	4,4-Dimethyl-cholesta-22,24-dien-5-ol		-		0.12	±	0.12
53.3	Stigmast-24(28)-en-3-one	0.34	±	0.11	0.72	±	0.16
53.4	Unidentified Steroid (149,177,355,391,430)	0.15	±	0.05	0.51	±	0.21
53.8	12-Oleanen-3-yl acetate		-		0.09	±	0.09
54.2	Stigmast-4-en-3-one	0.43	±	0.21	0.28	±	0.10
54.8	Lup-20(29)-en-3-ol, acetate		-		0.13	±	0.13
56.1	Lupan-3-ol, acetate		-		0.14	±	0.14
56.3	Lupan-3-ol, derivative?		-		0.19	±	0.19
56.9	Cholest-5-en-3-ol nonanoate	0.05	±	0.05		-	
57.5	Cholest-5-en-3-ol 9-octadecenoate	0.04	±	0.04		-	
59.2	Cholest-3-ene, derivative?	0.66	±	0.66	0.07	±	0.07
61.5	3-(Acetyloxy)-cholan-24-oic acid, methyl ester?		-		0.66	±	0.66
64.0	Stigmasta-5,22-dien-3-ol, acetate	0.32	±	0.32	0.16	±	0.16
67.6	Stigmast-5-en-3-ol, oleate	0.01	±	0.01	0.12	±	0.12
	Others:						
50.1	α-Tocopherol	4.33	±	0.77	2.25	±	0.33