Biochemical Systematics and Ecology 39 (2011) 95–101

Contents lists available at ScienceDirect

Biochemical Systematics and Ecology

journal homepage: www.elsevier.com/locate/biochemsyseco

New compounds, sexual differences, and age-related variations in the femoral gland secretions of the lacertid lizard *Acanthodactylus boskianus*

Eraqi R. Khannoon [a,b,*], Birte Flachsbarth [c], Afaf El-Gendy [b], Krysia Mazik [a], Jörg D. Hardege [a], Stefan Schulz [c]

[a] *Department of Biological Sciences, University of Hull, Cottingham Road, Hull HU6 7RX, UK*
[b] *Zoology Department, Faculty of Science, Fayoum University, El Fayoum, 63514, Egypt*
[c] *Technische Universität Braunschweig, Institut für Organische Chemie, Hagenring 30, 38106 Braunschweig, Germany*

ARTICLE INFO

Article history:
Received 18 November 2009
Accepted 15 January 2011
Available online 26 February 2011

Keywords:
Acanthodactylus boskianus
Femoral gland secretions
Glycerol monoethers
Monoglycerides
Sexual variability
Pheromones

ABSTRACT

Integumental gland secretions in lizards have been postulated to play a role as semiochemicals, but few studies have analysed the chemical nature of the gland secretions used in communication. We analysed the femoral gland secretions of *Acanthodactylus boskianus* using GC–MS, compared secretions of both sexes and different ages of males. For the first time in reptiles monoglycerides of fatty acids and glycerol monoethers of long chain alcohols were identified. In addition, alcohols, steroids, carboxylic acids, alkanes, amides, aldehydes, carboxylic acid esters, and squalene occurred. Sexual differences and age correlation in the amount of all major groups of compounds occurred, as such these results strengthen the theory that these secretions are used as semiochemicals. This work lays the foundations to test in future the role of chemical cues in mate choice and dominance hierarchies in lizards and to test the activity of compounds in behavioural assays to eventually identify the pheromones.

1. Introduction

Epidermal gland secretions of reptiles can have pheromonal properties (Cooper et al., 1994; Lopez et al., 1997) and may play an important role in sexual signalling and territorial scent marking (Alberts, 1993; Mason, 1992). Chemical signals are used in intraspecific communication between lizards such as the desert iguana (Alberts, 1992; Halpern, 1992; Mason, 1992). In several species the pheromonal activity of the femoral gland secretions has been shown (Alberts, 1993; Aragon et al., 2001; Cooper and Vitt, 1984; Lopez et al., 2006; Martin et al., 2007). The secretory activity of these glands varies seasonally and increases during the period of sexual activity. In some families of squamates only males have these glands (Alberts, 1993; Cole, 1966; El-Shershaby et al., 2006; Khannoon, 2004; Vanwyk, 1990).

Studies of the composition of the skin chemicals originating from these glands showed that they are composed of both lipids and proteins (Mason and Gutzke, 1990; Weldon and Bagnall, 1987). The major compounds involved in chemical communication were suggested to be of lipidic nature (Bull et al., 1999; Cooper and Garstka, 1987; LeMaster and Mason, 2001; Mason and Gutzke, 1990). On the other hand, the involvement of proteins cannot be discounted, given their importance in amphibians (Toyoda et al., 2004). Lipophilic classes of compounds are similar between species of lizards but there is interspecific variation in the presence and abundance of specific compounds and in the number of compounds in each species (Weldon et al., 2008).

* Corresponding author. Zoology Department, Faculty of Science, Fayoum University, Fayoum 63514, Egypt. Tel.: +20 846344264.
E-mail addresses: e.r.khannoon@hull.ac.uk, err00@fayoum.edu.eg (E.R. Khannoon).

0305-1978/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bse.2011.01.008

Acanthodactylus boskianus is a medium to large sized lizard, inhabiting deserts and semi-deserts in Africa. Like other lacertids, this species has an array of epidermal glands in both, males and females. These glands secrete semisolid plugs of secretions. Behavioural experiments examining the femoral secretions of *A. boskianus* (Khannoon et al., 2010; Khannoon, 2009) showed a sexual difference in the responses towards the secretions, which could be related to sexual differences in their chemical composition. Our hypothesis was that the composition of the femoral secretions of *A. boskianus* might be different between sexes and probably between males of different ages and that variability exists between individual males. We suggest that these secretions might compose characteristic profiles of compounds, typical for *A. boskianus*, and that the variability may play a role in mate choice and the establishment of dominance hierarchies, or in territorial marking. In the present study we report the results of the chemical analyses of individuals of *A.boskianus* and discuss these from a chemo-ecological perspective.

2. Materials and methods

2.1. Sample collection

A. boskianus individuals were captured from Balteem, Northern coast of Egypt, during April 2007 which coincides with the activity and mating season of these lizards. The animals were transferred to Hull University, UK, and kept for behaviour experiments. In order to test the effect of animal age on the chemical components of the secretions, we used 51 different males ranging from 4.80 cm to 7.60 cm snout-vent length (SVL) which is reflecting the different ages of the animals. For sex specificity we selected 16 adult males and 10 adult females that had the highest SVL within a very narrow range of SVL differences. Secretions were collected by gently squeezing the plugs from the femoral pores of the lizards using forceps. The secretion of each individual (8 mg) was collected directly into glass vials with Teflon-lined caps and then dissolved in 250 μL of dichloromethane (DCM) (Aldrich, GC grade). The collected samples were transferred to Braunschweig, Germany at −18 °C and kept until processing for analysis. Control samples with the solvent at the same conditions of collecting the secretion were used to exclude impurities.

2.2. Derivatization

Fifty μL of the liquid secretion was placed in a 2 ml vial and the solvent was removed in a gentle stream of nitrogen at 50 °C. The residue was taken up in 10 μL dichloromethane and 50 μL *N*-methyl–*N*-trimethylsilyltrifluoroacetamide (MSTFA) were added. The mixture was heated to 50 °C for 30–60 min in a vial with a closed cap. Then the solvent and the remaining reagent were evaporated in a gentle stream of nitrogen at 50 °C and the residue was taken up in 10 μL dichloromethane. Exactly 1.0 μL was injected into the gas chromatography–mass spectrometry (GC–MS) system.

2.3. GC–MS analysis

Samples were analysed using a Hewlett-Packard model 6890 gas chromatograph connected to a Hewlett-Packard model 5973 mass-selective detector equipped with a BPX-5 column: 25 m × 0.22 mm i.d., 0.25 μm film thickness (SGE). The temperature program was as follows: 50 °C for 5 min, then with 5 °C/min to 320 °C, 30 min hold time. Helium was used as carrier gas with 1 ml/min in constant flow mode. Accelerating voltage of MS was 70 eV. Compounds were identified by comparison of mass spectra and retention indices of derivatized and underivatized samples with those of reference compounds.

2.4. Data analysis

The relative amount of each component was determined as the percent of the total ion current (TIC). Chemicals included as variables in the statistical analysis were only those which were presented in all individuals and constituted >0.1% of TIC. The relative areas of the selected peaks were transformed according to Aitchison (1986) following the formula $Z_{ij} = \ln (Y_{ij}/g(Y_j))$, where Z_{ij} is the standardised peak area i for individual j, Y_{ij} is the peak area i for individual j, and $g(Y_j)$ is the geometric mean of all peaks for individual j. These transformed areas were used as dependent variables in a multivariate analysis of variance (MANOVA). This test was used to determine if males are different from females in the abundances of the secretion chemicals. To detect which chemicals responsible for the differences we applied one-way ANOVA on each chemical. Correlation analysis (Pearson correlation) test was used to investigate the relationship between the chemical abundance and size (SVL) of the male lizards. The transformed areas of these chemicals were used as the dependent variables in the test.

3. Results

The same compounds were detected in the total ion chromatogram of both male and female *A. boskianus* secretions. No two individuals had the same quantitative composition of the compounds. A total of 122 chemicals were identified in both sexes (Table 1). The identified compounds were steroids, alcohols, carboxylic acids, alkanes, amides, aldehydes, carboxylic acid esters, and squalene. These compounds were identified using standard procedures. The GC–MS of the underivatized natural extracts showed the presence of more polar compounds, which could not be identified because of their poor elution properties. Therefore the extracts were analysed after derivatization with MSTFA to form trimethylsilyl-derivatives, thus enabling the

Table 1
List of compounds identified in at least one sample of an individual *A. boskianus*. The gas chromatographic retention index (RI) is also given. Alcohols and acids were detected as the respective trimethylsilylated compounds, thus the RI shown is that of the respective derivative. The relative amount of each component was determined as the percent of the total ion current (TIC) and reported as the average ± standard error.

RI	Compound	Males ($N = 16$)	Females ($N = 10$)
1058	2-Hydroxypropanoic acid	0.05 ± 0.02	0.11 ± 0.01
1076	Hexanoic acid	Traces	Traces
1077	1,2-Dihydroxypropane	Traces	Traces
1116	Nonanal	0.05 ± 0.01,	0.03 ± 0.09
1216	Decanal	Traces	Traces
1265	Glycerol	0.60 ± 0.11	0.72 ± 0.08
1320	2,3-Dihydroxypropanoic acid	Traces	Traces
1360	Nonanoic acid	0.05 ± 0.01	0.04 ± 0.01
1400	Tetradecane	Traces	Traces
1458	Decanoic acid	0.14 ± 0.09	0.22 ± 0.04
1490	Hydrocarbon	Traces	Traces
1500	Pentadecane	Traces	Traces
1507	Hexanedioic acid	Traces	Traces
1530	Methyl dodecanoate	0.01 ± 0.09	0.02 ± 0.05
1560	Hydrocarbon	0.02 ± 0.08	0.04 ± 0.05
1562	1-Dodecanol	0.03 ± 0.01	0.07 ± 0.02
1600	Hexadecane	0.01 ± 0.02	0.09 ± 0.08
1630	Isopropyl dodecanoate	Traces	Traces
1645	Hydrocarbon	Traces	Traces
1653	Dodecanoic acid	0.05 ± 0.02	0.07 ± 0.03
1700	Heptadecane	0.03 ± 0.09	0.07 ± 0.12
1703	Hydrocarbon	Traces	Traces
1725	Unknown compound	0.21 ± 0.18	0.25 ± 0.24
1731	Hydrocarbon	0.05 ± 0.01	0.07 ± 0.06
1755	1-Tetradecanol	0.02 ± 0.02	0.09 ± 0.05
1800	Octadecane	0.02 ± 0.01	0.04 ± 0.09
1811	12-Methyltridecanoic acid	0.03 ± 0.02	0.04 ± 0.06
1849	Tetradecanoic acid	0.08 ± 0.09	0.10 ± 0.05
1900	Nonadecane	0.32 ± 0.06	0.63 ± 0.09
1910	13-Methyltetradecanoic acid	0.16 ± 0.04	0.07 ± 0.03
1917	12-Methyltetradecanoic acid	0.07 ± 0.09	0.08 ± 0.02
1933	Methyl hexadecanoate	0.23 ± 0.12	0.56 ± 0.35
1943	Hydrocarbon	Traces	Traces
1947	Pentadecanoic acid	0.07 ± 0.01	0.22 ± 0.01
1956	1-Hexadecanol	0.07 ± 0.02	0.67 ± 0.10
1981	14-Methylpentadecenoic acid	0.07 ± 0.01	0.12 ± 0.02
2000	Eicosane	0.07 ± 0.09	0.37 ± 0.16
2008	14-Methylpentadecanoic acid	0.08 ± 0.09	0.21 ± 0.03
2023	Hexadecenoic acid	0.45 ± 0.15	1.42 ± 0.06
2044	Hexadecanoic acid	2.38 ± 0.44	0.57 ± 0.15
2100	Heneicosane	0.51 ± 0.07	0.87 ± 0.07
2105	15-Methylhexadecanoic acid	0.15 ± 0.09	0.08 ± 0.05
2114	14-Methylhexadecanoic acid	Traces	Traces
2116	Heptadecenoic acid	0.53 ± 0.07	1.08 ± 0.21
2134	Methyl octadecanoate	0.32 ± 0.09	0.85 ± 0.12
2143	Heptadecanoic acid	0.13 ± 0.10	0.45 ± 0.07
2150	1-Octadecanol	0.85 ± 0.06	0.20 ± 0.03
2200	Docosane	0.74 ± 0.06	1.05 ± 0.06
2213	3-Hydroxyhexadecanoic acid	0.41 ± 0.15	0.48 ± 0.26
2216	Octadecadienoic acid	0.41 ± 0.09	0.22 ± 0.09
2223	Octadecenoic acid	2.38 ± 0.40	0.35 ± 0.13
2242	Octadecanoic acid	2.57 ± 0.47	0.33 ± 0.07
2267	*N*,*N*-Dimethylhexadecanamide	0.47 ± 0.07	0.82 ± 0.15
2300	Tricosane	0.94 ± 0.08	1.54 ± 0.09
2302	2-Octadecenoic acid	Traces	Traces
2322	1,3-Octadecanediol	0.11 ± 0.04	0.16 ± 0.08
2340	Nonadecanoic acid	0.16 ± 0.12	0.21 ± 0.04
2348	1-Eicosanol	0.69 ± 0.08	0.42 ± 0.18
2377	Glycerol 1-pentadecyl ether	0.04 ± 0.01	0.05 ± 0.04
2387	Glycerol 1-tetradecanoate	0.24 ± 0.17	0.21 ± 0.31
2398	3-Hydroxyoctadecanoic acid	Traces	Traces
2400	Tetracosane	Traces	Traces
2420	Eicosenoic acid	0.32 ± 0.91	0.54 ± 0.07
2441	Eicosanoic acid	0.56 ± 0.10	0.31 ± 0.08
2441	*N*,*N*-Dimethyloctadecadieneamide	Traces	Traces
2473	Glycerol 1-hexadecyl ether	0.31 ± 0.21	0.42 ± 0.36
2478	*N*,*N*-Dimethyloctadecaneamide	0.32 ± 0.06	0.48 ± 0.18

(*continued on next page*)

Table 1 (*continued*)

RI	Compound	Males ($N = 16$)	Females ($N = 10$)
2500	2-Eicosenoic acid	Traces	Traces
2500	Pentacosane	0.87 ± 0.18	3.02 ± 1.02
2513	1.3-Eicosanediol	0.03 ± 0.08	0.05 ± 0.89
2539	Heneicosanoic acid	0.06 ± 0.05	0.07 ± 0.47
2545	1-Docosanol	2.87 ± 0.23	4.29 ± 1.19
2568	Glycerol 1-heptadecyl ether	0.15 ± 0.56	0.20 ± 0.82
2580	Glycerol 1-hexadecanoate	0.52 ± 0.72	0.72 ± 0.93
2590	3-Hydroxyicosanoic acid	0.13 ± 0.05	0.16 ± 0.07
2600	Hexacosane	0.89 ± 0.03	1.57 ± 0.93
2612	Docosenoic acid	0.04 ± 0.12	0.05 ± 0.06
2637	Docosanoic acid	0.28 ± 0.02	0.38 ± 0.04
2642	1-Tricosanol	0.22 ± 0.02	0.33 ± 0.06
2663	Glycerol 1-octadecyl ether	1.04 ± 0.04	1.76 ± 0.02
2700	Heptacosane	1.01 ± 0.79	2.17 ± 0.85
2709	1,3-Docosanediol	Traces	Traces
2716	Tetracosen-1-ol	0.13 ± 0.67	0.16 ± 0.95
2741	1-Tetracosanol	8.87 ± 0.50	8.76 ± 1.19
2745	Glycerol 1-octadecadienoate	1.02 ± 0.98	1.31 ± 0.07
2750	Glycerol 1-octadecenoate	1.71 ± 0.32	1.94 ± 0.71
2760	Glycerol 1-nonadecyl ether	0.39 ± 0.72	0.45 ± 0.09
2772	Glycerol 1-octadecanoate	0.54 ± 0.06	0.67 ± 0.18
2792	Glycerol 1-(3-hydroxy) octadecyl ether	0.37 ± 0.09	0.39 ± 0.28
2800	Octacosane	0.56 ± 0.06	1.72 ± 1.09
2814	Squalene	0.85 ± 0.45	0.98 ± 0.65
2834	Tetracosanoic acid	0.09 ± 0.04	0.05 ± 0.03
2838	1-Pentacosanol	0.68 ± 0.06	0.57 ± 0.05
2854	Glycerol 1-eicosyl ether	1.28 ± 0.24	0.50 ± 0.09
2900	Nonacosane	0.52 ± 0.08	0.86 ± 0.52
2913	Hexacosen-1-ol	Traces	Traces
2935	1-Hexacosanol	25.15 ± 0.94	18.09 ± 1.92
2948	Glycerol 1-eicosanoate	0.32 ± 0.18	0.65 ± 0.07
2977	Glycerol 1-(3-hydroxy)eicosyl ether	0.15 ± 0.06	0.21 ± 0.05
3000	Triacontane	0.28 ± 0.06	0.56 ± 0.15
3024	Glycerol 1-docosenyl ether	0.14 ± 0.02	0.18 ± 0.04
3033	1-Heptacosanol	0.56 ± 0.04	0.39 ± 0.05
3045	Glycerol 1-docosyl ether	0.23 ± 0.02	0.52 ± 0.07
3059	Steroid $M = 386$	0.02 ± 0.01	0.05 ± 0.03
3067	Steroid $M = 388$	0.02 ± 0.01	0.03 ± 0.02
3094	Steroid $M = 386$	0.09 ± 0.04	0.08 ± 0.05
3103	Hentriacontane	0.15 ± 0.04	0.19 ± 0.01
3103	Steroid $M = 384$	Traces	Traces
3116	Octacosen-1-ol	0.08 ± 0.04	1.01 ± 0.21
3133	1-Octacosanol	7.51 ± 0.64	3.80 ± 0.71
3141	Cholesterol	30.78 ± 1.80	26.28 ± 2.09
3159	Cholestan-3-ol	0.43 ± 0.11	0.52 ± 0.09
3190	Cholesta-5,7-dien-3-ol	1.26 ± 1.39	0.39 ± 0.08
3200	Dotriacontane	0.32 ± 0.01	0.54 ± 0.11
3231	1-Nonacosanol	0.21 ± 0.05	0.18 ± 0.07
3247	Campesterol	1.58 ± 0.19	1.77 ± 0.26
3269	Stigmasterol	1.87 ± 0.08	1.21 ± 0.53
3300	Tritriacontane	Traces	Traces
3330	1-Triacontanol	0.65 ± 0.05	0.89 ± 0.03
3332	β-Sitosterol	0.55 ± 0.32	0.96 ± 0.08
3400	Tetratriacontane	0.04 ± 0.01	0.09 ± 0.04
3528	1-Dotriacontanol	0.05 ± 0.01	0.02 ± 0.01

GC–MS analysis of the more polar compounds. This method led to the identification of additional components not previously reported from lizards (Fig. 1). Monoglycerides carrying the carboxylic acid at C-1 were identified by their characteristic ions at *m/z* M-103 (M-$(CH_3)_3SiOCH_2^+$), 103 ($(CH_3)_3SiOCH_2^+$) and 205 ($(CH_3)_3SiOCH_2(CH_3)_3SiOCH^+$) (Fig. 2C). Related monoethers of glycerol connected at C-1 show a characteristic base peak at *m/z* 205 which is of low abundance in respective monoglycerides. Furthermore, an ion *m/z* M-147 (M-$(CH_3)_3SiO(CH_3)_2$) is characteristic together with M-205-2 H (Fig. 2D). Furthermore, 1, 3-alkanediols were identified by the ions *m/z* 103 ($(CH_3)_3SiOCH_2^+$), 219 ($(CH_3)_3SiOCH_2CH_2(CH_3)_3SiOCH^+$), and M-117 (M-$(CH_3)_3SiOCH_2CH_2^+$) (Fig. 2B). All these identifications were verified by comparison with synthetic reference compounds. The fourth class of compounds not reported from lizards before are respective glycerol ethers of the alkanediols, connected terminally at both alcohols. Their mass spectra indicated the presence of three trimethylsilyl groups. The position of the silyloxy group in the long chain is indicated by a characteristic ion, e.g. *m/z* 313 in the mass spectra shown in Fig. 2A, while the other ions can be explained as before. No synthetic reference compound existed for this ether, so that this identification can so far only be regarded as tentative.

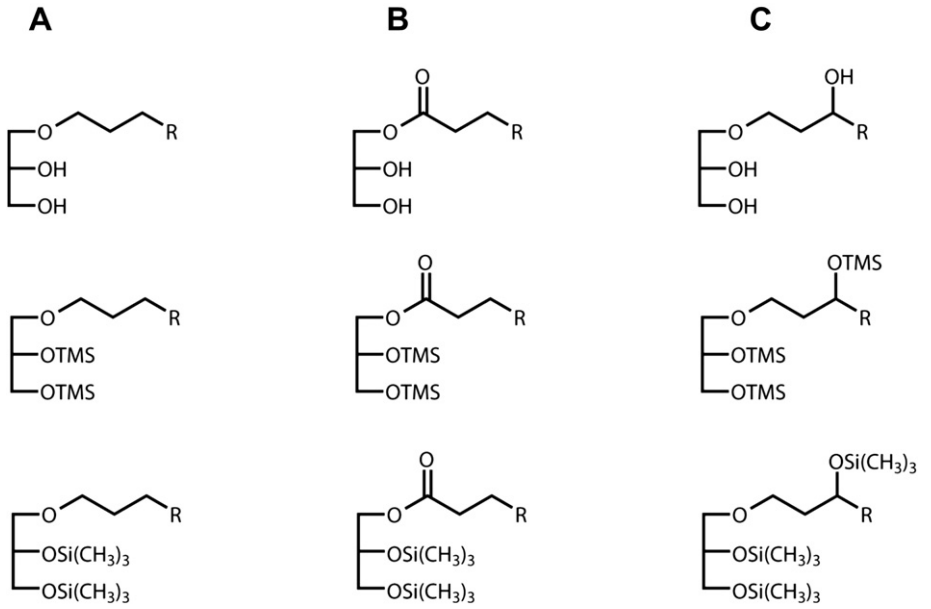

Fig. 1. The structural formula of the newly recorded compounds from the femoral gland secretions of *Acanthodactylus boskianus*. Monoether of glycerol (A), monoglyceride (B), glycerol ether of alkanediol (C). Each group is shown as natural structure (top), and trimethylsilyl (TMS) derivative (-$Si(CH_3)_3$), middle and bottom.

The major compound groups present in the secretion of adult males were alcohols (50.29% of the TIC), steroids (35.54%), carboxylic acids (9.37%), glycerol monoethers (3.11%), and monoglycerides (1.21%). The other classes occurred only in minor amounts. The most abundant chemical detected was cholesterol (30.78%). The 20 chosen compounds were used as dependent variables in the statistical analysis after transformation of their peak areas. Multivariate analysis showed that there are significant differences between males and females on the combined dependent variables: (MANOVA; Wilks' $\lambda = 0.022$, $F_{20,5} = 11.26$, $P = 0.007$). One-way ANOVA showed that males have significant higher proportions of eicosanoic acid ($F_{1,24} = 5.26$, $P = 0.031$), octadecanoic acid ($F_{1,24} = 7.21$, $P = 0.013$), 1-octacosanol ($F_{1,24} = 4.85$, $P = 0.038$), 1-hexacosanol ($F_{1,24} = 4.36$, $P = 0.048$), 1-octadecanol ($F_{1,24} = 4.80$, $P = 0.038$), glycerol 1-eicosyl ether ($F_{1,24} = 15.01$, $P = 0.001$), and cholesterol ($F_{1,24} = 5.09$, $P = 0.033$). On the other hand, females have higher proportions of heptadecenoic acid ($F_{1,24} = 13.13$, $P = 0.001$), 1-docosanol ($F_{1,24} = 8.34$, $P = 0.008$), and β-sitosterol ($F_{1,24} = 5.75$, $P = 0.025$). Pearson correlation data showed significant positive correlation between the male size and hexadecanoic acid ($r = 0.52$, $P = 0.001$), 1-octadecanol ($r = 0.49$, $P = 0.001$), and cholesterol ($r = 0.41$, $P = 0.003$). On the other hand, negative correlations were detected between size of the male and heptadecenoic acid ($r = -0.36$, $P = 0.01$), 1-octacosanol ($r = -0.34$, $P = 0.02$), β-sitosterol ($r = -0.29$, $P = 0.04$), and campesterol ($r = -0.32$, $P = 0.02$).

4. Discussion

Several components of *A. boskianus* have been identified here for the first time in the femoral gland secretions of lizards. These compounds are monoglycerides and glycerol monoethers which have been found in both males and females. Glycerol alkyl monoethers have been rarely identified from nature, e.g. in marine sponges (Quijano et al., 1994), bacteria (Ring et al., 2006), starfish (Snyder et al., 1969), clams and mussels (Hanuš et al., 2009), octopus (Jahnke et al., 2001), or rats (Paltauf and Polheim, 1970). The unusual glycerol 1-(3-hydroxy) alkyl ethers have not been reported before form nature with glycerol 1-eicosyl ether showing higher abundance in males.

Alcohols are found regularly in femoral gland secretions and were reported as major components in *Acanthodactylus erythrurus* (Lopez and Martin, 2005). The high percentage of alcohols makes them potentially ideal compounds to function as chemical fingerprint compounds of the *Acanthodactylus* genus. 1-octadecanol showed high sexual variability and is positively correlated to the size of males. This suggests that 1-octadecanol could potentially be used as a chemosignal in *A. boskianus*, and also supports the theory of its role as dominance badge in lacertid lizard *Lacerta monticola* (Martin et al., 2007).

Steroids represented a high percent of the secretions lipids. Escobar et al. (2003) postulated that cholesterol and the protein part of secretions function as fixatives for semiochemicals, or as controlled-release carrier through helping to constitute an unreactive, a polar matrix that delivers the compounds that are the true semiochemicals. In our results, cholesterol correlated to the size and therefore possibly the age of males. This suggests that it potentially reflects the strength and physical quality of a male, which are important criteria in dominance detection and mate choice (Martin and Lopez, 2006). On the other hand, lower proportions of β-sitosterol and campesterol might be an indication of male fitness as both are higher in younger males.

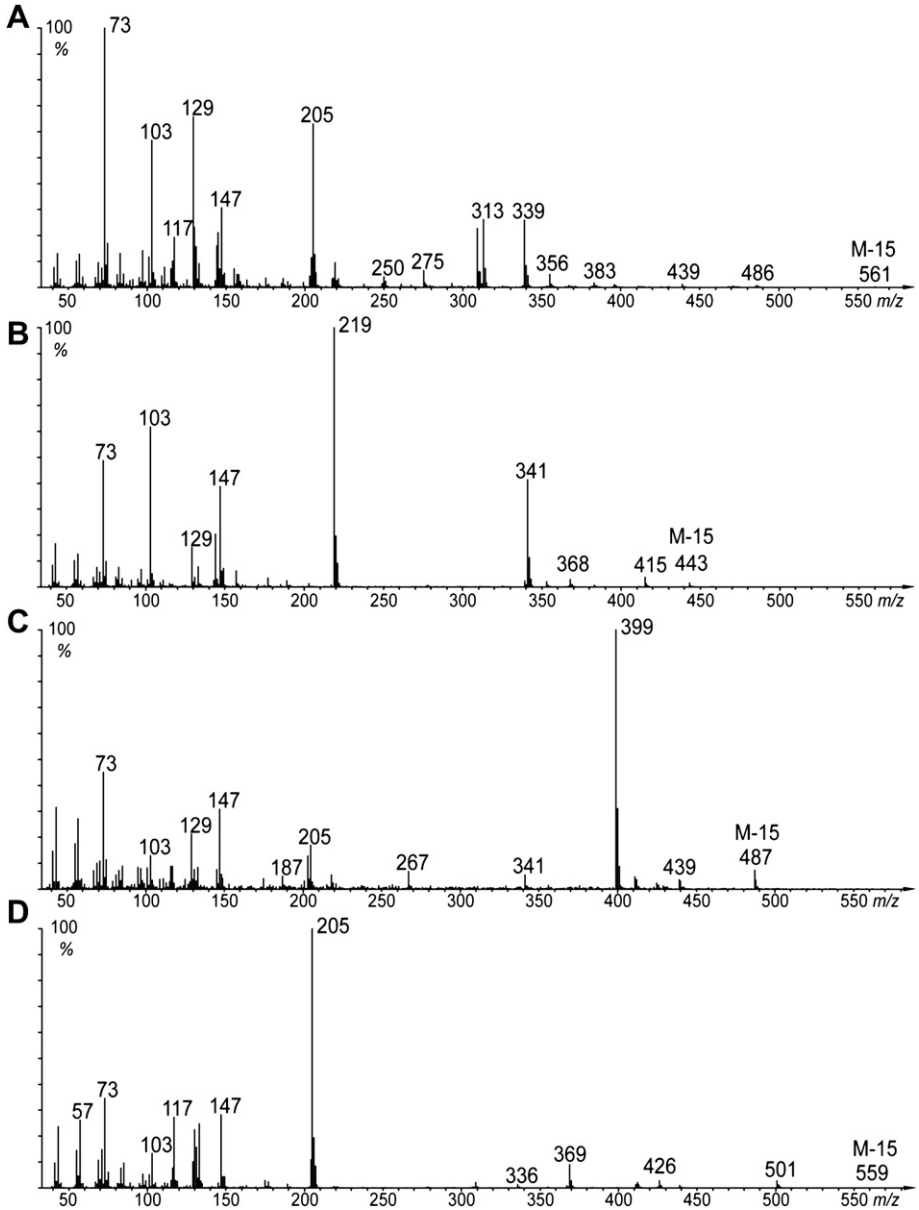

Fig. 2. Mass spectra of the trimethylsilyl derivatives of compounds not reported before from lizards; (A) Glycerol ether of alkanediol (Glycerol 1-(3-hydroxy) octadecyl ether), (B) 1,3-Alkanediol (1,3-Eicosanediol), (C) Monoglyceride (Glycerol 1-octadecanoate), (D) Monoether of glycerol (Glycerol 1-eicosyl ether).

In the present study, carboxylic acids found were ranging between C_3 and C_{24}. The presence of fatty acids (tetradecanoic, hexadecanoic, octadecanoic, and octadecenoic acids) normally present in internal tissue (Nicolaides, 1974), confirms the holocrine secretion of these femoral glands (Khannoon, 2004). Different to other lizard groups, the lacertid lizard femoral secretion; *Acanthodactylus*, *Liolaemus* (Escobar et al., 2003), and *Lacerta* (Aragon et al., 2001) contains short chain acids (3–6 carbon atoms), probably specific for lacertids. In our study, heptadecenoic octadecanoic, and eicosanoic acids showed sexual variability, while hexadecanoic acid is more abundant in older males. These long chain carboxylic acids are compounds of low volatility, which could be important for the persistence of scent markings of territorial males. Alberts (1990) reported that proteins could be used in individual and sex recognition. Additional roles for both the proteins and the lipids of the secretion might be that the proteins are reducing the evaporation rate by providing a matrix. Such a role was proved by Humphries et al. (1999) for major urinary proteins (MUPS) which slow the release of volatiles in the urine of the house mice *Mus domesticus* enabling mice to use these as long-term territorial markers.

In conclusion, the femoral gland secretions of *A. boskianus* contain different classes of compounds, shared by other reptiles, and some specific compounds such as glycerol monoethers and monoglycerides which are recorded here for the first time. Nevertheless, it seems likely that these compounds will also be found in the secretion of other lizards if appropriate methods

for their detection like silylation are performed. Some detected compounds showed sexual variation while some also varied with the size, making them potential candidates for a role as chemosignal in *A. boskianus*. The presented chemical data confirms our behavioural work on the same species, which showed a sexual difference in response towards the femoral gland secretions (Khannoon et al., 2010; Khannoon, 2009). Femoral gland secretions are as such a potential source of chemical information about an individual ranging from sex, health status, dominance status, to age, all of this being key elements in mate choice and territorial marking. Examining the behavioural functions using fractions of femoral gland secretions should clarify whether they are used as pheromones.

Acknowledgements

We thank Prof. M. Bahgat for his help in the proposal set up. Financial support was received from the Ministry of Higher Education, Egypt. Special thanks go to Victor Swetez for technical support in lizard culture and Maggy Harley for technical support with GC–MS.

References

Alberts, A.C., 1990. Chemical properties of femoral gland secretions in the desert Iguana, *Dipsosaurus dorsalis*. J. Chem. Ecol. 16, 13–25.
Alberts, A.C., 1992. Pheromonal self-recognition in desert Iguanas. Copeia, 229–232.
Alberts, A.C., 1993. Chemical and behavioural studies of femoral gland secretions in iguanid lizards. Brain Behav. Evolut. 41, 255–260.
Aragon, P., Lopez, P., Martin, J., 2001. Discrimination of femoral gland secretions from familiar and unfamiliar conspecifics by male Iberian rock-lizards, *Lacerta monticola*. J. Herpetol. 35, 346–350.
Aitchison, J., 1986. The Statistical Analysis of Compositional Data: Monographs in Statistics and Applied Probability. Chapman and Hall, London.
Bull, C.M., Griffin, C.L., Johnston, G.R., 1999. Olfactory discrimination in scat piling lizards. Behav. Ecol. 10, 136–140.
Cole, C., 1966. Femoral glands in lizards: a review. Herpetologica 22, 199–206.
Cooper, W.E., Garstka, W.R., 1987. Lingual responses to chemical fractions of urodaeal glandular pheromone of the skink *Eumeces laticeps*. J. Exp. Zool. 242, 249–253.
Cooper, W.E., Lopez, P., Salvador, A., 1994. Pheromone detection by an amphisbaenian. Anim. Behav. 47, 1401–1411.
Cooper, W.E., Vitt, L.J., 1984. Conspecific odor detection by the male broad-headed skink, *Eumeces laticeps* - effects of sex and site of odor source and of male reproductive condition. J. Exp. Zool. 230, 199–209.
El-Shershaby, A.M., Mohammed, M.B.H., Khannoon, E.R., 2006. Histological survey on the epidermal glands in some lizards. Egypt. J. Zool 46, 105–118.
Escobar, C.M., Escobar, C.A., Labra, A., Niemeyer, H.M., 2003. Chemical composition of precloacal secretions of two *Liolaemus fabiani* populations: are they different? J. Chem. Ecol. 29, 629–638.
Halpern, M., 1992. Nasal Chemical Senses in Reptiles: Structure and Function. In: Cooper, W., Greenberg, N., Gans, C., Crews, D. (Eds.), Biology of the Reptilia. University Of Chicago Press, Chicago, pp. 423–523.
Hanuš, L., Levitsky, D., Shkrob, I., Dembitsky, V., 2009. Plasmalogens, fatty acids and alkyl glyceryl ethers of marine and freshwater clams and mussels. Food Chem. 116, 491–498.
Humphries, R.E., Robertson, D.H.L., Beynon, R.J., Hurst, J.L., 1999. Unravelling the chemical basis of competitive scent marking in house mice. Anim. Behav. 58, 1177–1190.
Jahnke, L.L., Eder, W., Huber, R., Hope, J.M., Hinrichs, K.U., Hayes, J.M., Des Marais, D.J., Cady, S.L., Summons, R.E., 2001. Signature lipids and stable carbon isotope analyses of octopus spring hyperthermophilic communities compared with those of Aquificales representatives. Appl. Environ. Microb. 67, 5179–5189.
Khannoon, E.R., 2004. Functional anatomy of the skin in some lizards. M.Sc. Dissertation, Biology Department, Cairo University, Egypt.
Khannoon, E.R., 2009. Comparative chemical ecology, behaviour, and evolutionary genetics of *Acanthodactylus boskianus* (Reptilia: Lacertidae). Ph.D. Dissertation, The University of Hull, UK.
Khannoon, E.R., El–Gendy, A., Breithaupt, T., Hardege, J.D., 2010. Sexual differences in behavioural response to femoral gland pheromones of *Acanthodactylus boskianus* (Squamata: Lacertidae). Herpetol. J 20, 225–229.
LeMaster, M., Mason, R., 2001. Annual and seasonal variation in the female sexual attractiveness pheromone of the red-sided garter snake, *Thamnophis sirtalis parietalis*. In: Marchlewska-Koj, A., Lepri, J., Müller-Schwarze, D. (Eds.), Chemical Signals in Vertebrates 9. Kluwer Academic/Plenum Publishers, New York, pp. 369–376.
Lopez, P., Salvador, A., Cooper, W.E., 1997. Discrimination of self from other males by chemosensory cues in the amphisbaenian (*Blanus cinereus*). J. Comp. Psych. 111, 105–109.
Lopez, P., Martin, J., 2005. Age related differences in lipophilic compounds found in femoral gland secretions of male spiny-footed lizards. *Acanthodactylus erythrurus*. Z. Naturforsch C 60, 915–920.
Lopez, P., Amo, L., Martin, J., 2006. Reliable signaling by chemical cues of male traits and health state in male lizards, *Lacerta monticola*. J. Chem. Ecol. 32, 473–488.
Martin, J., Lopez, P., 2006. Age-related variation in lipophilic chemical compounds from femoral gland secretions of male lizards *Psammodromus algirus*. Biochem. Syst. Ecol. 34, 691–697.
Martin, J., Moreira, P.L., Lopez, P., 2007. Status-signalling chemical badges in male Iberian rock lizards. Funct. Ecol. 21, 568–576.
Mason, R., 1992. Reptilian pheromones. In: Cooper, W., Greenberg, N., Gans, C., Crews, D. (Eds.), Biology of the Reptilia, vol. 18. University of Chicago Press, Chicago, Chicago, pp. 114–228.
Mason, R.T., Gutzke, W.H.N., 1990. Sex recognition in the leopard gecko, *Eublepharis macularius* (Sauria, Gekkonidae) possible mediation by skin-derived semiochemicals. J. Chem. Ecol. 16, 27–36.
Nicolaides, N., 1974. Skin lipids, their biochemical uniqueness. Science 186, 19–26.
Paltauf, F., Polheim, D., 1970. Occurrence of C20-alk-1-enyl and C20-alkyl glycerol ethers in phospholipids of rat intestinal mucosa. Biochim. Biophys. Acta 210, 187–189.
Quijano, L., Cruz, F., Navarrete, I., Gomez, P., Rios, T., 1994. Alkyl glycerol monoethers in the marine sponge *Desmapsamma anchorata*. Lipids 29, 731–734.
Ring, M.W., Schwar, G., Thiel, V., Dickschat, J.S., Kroppenstedt, R.M., Schulz, S., Bode, H.B., 2006. Novel iso-branched ether lipids as specific markers of developmental sporulation in the *Myxobacterium myxococcus xanthus*. J. Biol. Chem. 281, 36691–36700.
Snyder, F., Malone, B., Blank, M.L., 1969. The biosynthesis of alkyl glyceryl ethers by microsomal enzymes of digestive glands and gonads of the starfish, *Asterias forbesi*. Biochim. Biophys. Acta 187, 302–306.
Toyoda, F., Yamamoto, K., Iwata, T., Hasunuma, I., Cardinali, M., Mosconi, G., Polzonetti-Magni, A.M., Kikuyama, S., 2004. Peptide pheromones in newts. Peptides 25, 1531–1536.
Vanwyk, J.H., 1990. Seasonal testicular activity and morphometric variation in the femoral glands of the lizard *Cordylus polyzonus polyzonus* (Sauria, Cordylidae). J. Herpetol. 24, 405–409.
Weldon, P.J., Bagnall, D., 1987. A survey of polar and nonpolar skin lipids from lizards by thin-layer chromatography. Comp. Biochem. Phys. B 87, 345–349.
Weldon, P.J., Flachsbarth, B., Schulz, S., 2008. Natural products from the integument of nonavian reptiles. Nat. Prod. Rep. 25, 738–756.

ID	Title	Pages
1354340	New compounds, sexual differences, and age-related variations in the femoral gland secretions of the lacertid lizard Acanthodactylus boskianus	7