Chemical Compounds from the Preanal Gland Secretions of the Male Tree Agama (*Acanthocercus atricollis*) (Fam. Agamidae)

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Chemical signals have an important role in the reproductive behaviour of many lizards. However, the compounds secreted by their femoral or preanal glands, which may be used as sexual signals, are mainly known for lizard species within the Scleroglossa clade, whereas compounds in secretions of lizards within the Iguania clade are much less studied. Based on mass spectra, obtained by GC-MS, we found 60 lipophilic compounds in preanal gland secretions of the male tree agama (*Acanthocercus atricollis*) (fam. Agamidae), including steroids (mainly cholesterol, cholest-3-ene, and some of their derivatives), fatty acids ranging between $n\text{-C}_{12}$ and $n\text{-C}_{18}$ (mainly hexadecanoic and octadecenoic acids), ketones from $n\text{-C}_{17}$ to $n\text{-C}_{25}$, and other minor compounds, such as tocopherol, squalene, waxy esters, and furanones. We compare the compounds found with those present in other lizard species and discuss their potential function in social behaviour.

Key words: Steroids, Fatty Acids, Ketones, Lizards

Introduction

Males of many lizard species have conspicuous femoral or preanal glands that secrete chemical compounds especially during the reproductive season (Alberts, 1993; Weldon *et al.*, 2008). These compounds are important in intraspecific communication (Mason, 1992; Mason and Parker, 2010; Martín and López, 2011) as they may signal the characteristics and health state of a male (López *et al.*, 2006; Martín *et al.*, 2007a; López and Martín, 2012). This information may be used by females to select males (*e.g.* Martín and López, 2000, 2006a; López *et al.*, 2002, 2003; Olsson *et al.*, 2003; López and Martín, 2005a), or by other males to assess the fighting ability or dominance status of the sender (Aragón *et al.*, 2001; Carazo *et al.*, 2007; Martín *et al.*, 2007b).

Femoral or preanal gland secretions are composed of both lipids and proteins, but some lipophilic compounds seem to be involved in pheromonal communication (Mason, 1992; Martín and López, 2006a, 2010a, 2011). However, the presence and abundance of specific compounds varies widely between species (Weldon *et al.*, 2008), which might be due to phylogenetic or environmental differences (Alberts, 1992; Escobar *et al.*, 2003; Martín and López, 2006b). Most information on

the chemical composition of secretions relates to lizard species within the Scleroglossa clade, which are considered to rely mainly on chemical senses (Cooper, 1995). Lipophilic compounds have been described in secretions of European lizards of the family Lacertidae (López and Martín, 2005b, c, 2006, 2009; Martín and López, 2006c, d, 2010b; Gabirot *et al.*, 2008, 2010; Kopena *et al.*, 2009), an African lacertid (Khannoon *et al.*, 2011), an African cordylid (Louw *et al.*, 2007), and an American teiid (Martín *et al.*, 2011). In contrast, compounds in secretions of lizards within the Iguania clade have been less studied, probably because these lizards seem to rely more on visual cues, although many species have femoral or preanal glands and are capable of chemosensory conspecific recognition (Mason and Parker, 2010). Lipophilic compounds in gland secretions have been described in an iguanid (Weldon *et al.*, 1990; Alberts *et al.*, 1992a, b) and several South American tropidurids (Escobar *et al.*, 2001, 2003). In agamids, only an old study using thin-layer chromatography (TLC) analysed compounds in preanal gland secretions of the lizard *Uromastyx hardwickii*, suggesting the presence of fatty acids, triacylglycerols, wax esters, sterols and their esters, and phospholipids (Chauhan, 1986). To understand what determines

the composition of gland secretions of lizards and which is the role of the compounds in social behaviour, we need more studies that deal with a wider range of lizard species within different taxonomic groups and a larger variety of environmental conditions.

In the present paper, we report the results of an analysis by gas chromatography-mass spectrometry (GC-MS) of the lipophilic fraction of preanal gland secretions of the male blue-headed tree agama (*Acanthocercus atricollis*) from a Tanzanian population. This is an agamid lizard whose range extends from Ethiopia to the eastern and northern parts of South Africa (Spawls *et al.*, 2002; Wagner *et al.*, 2012). Tree agamas are large (average total length between 20 and 30 cm), diurnal, insectivorous, tree-dwelling lizards that are sexually dimorphic in size and colouration (Reaney and Whiting, 2002, 2003). Mature males have a bright blue head and two rows of 10–12 preanal pores each (Spawls *et al.*, 2002). Tree agamas spent most of their time on tree trunks, and only come to the ground to cross to another tree. They are strongly territorial and live in structured colonies with one dominant male, several females, and juveniles (Reaney and Whiting, 2002; Spawls *et al.*, 2002). We hypothesized that compounds secreted by preanal glands might be used to scent mark territories on tree trunks, having a role in social organization of this lizard.

Material and Methods

We obtained seven adult male tree agamas from a commercial dealer (Euroreptiles, L'Hospitalet, Barcelona, Spain). The lizards had been recently captured in the surroundings of Dodoma (Tanzania) during April 2012. Secretion samples were collected in Madrid immediately after arriving (May 19, 2012). We extracted secretions from preanal glands of male lizards by gently pressing with forceps around the preanal pores, and collected secretions directly with glass vials with glass inserts, which were later closed with Teflon-lined stoppers. Vials were stored at -20°C until analyses. We also used the same procedure but without collecting secretion, to obtain blank control vials that were treated in the same manner to compare with the lizards samples, and to be sure of excluding contaminants from the handling procedure and to be able to examine impurities in the solvent.

We analysed samples with a ThermoQuest (Austin, TX, USA) Trace 2000 gas chromatograph fitted with a poly(5% diphenylsiloxane/95% dimethylsiloxane) column (Supelco Equity-5, 30 m length x 0.25 mm ID, 0.25 μm film thickness; Supelco Co., Bellefonte, PA, USA) and a ThermoQuest Trace 2000 mass spectrometer as detector. Sample injections of 2 μl of each sample dissolved in 2 ml of *n*-hexane (capillary GC grade; Sigma-Aldrich Chemical Co., St. Louis, MO, USA) were performed in the splitless mode using helium as the carrier gas, with injector and detector temperatures at 250°C and 280°C , respectively. The oven temperature program was as follows: 50°C isothermal for 3 min, then increased to 300°C at a rate of $5^{\circ}\text{C}/\text{min}$, and then isothermal (300°C) for 15 min. Mass spectral fragments below $m/z = 46$ were not recorded. Impurities identified in the solvent and/or the control vial samples are not reported. Initial identification of secretion components was performed by comparison of sample mass spectra with those in the NIST/EPA/NIH (NIST 02) computerized mass spectral library. When possible, identifications were confirmed by comparison of spectra and retention times with those of authentic standards (from Sigma-Aldrich). The relative amount of each compound was determined as the percentage of the total ion current (TIC) using the Qual Browser module of the Xcalibur 1.2 software (Thermo Electron Finnigan Co., Austin, TX, USA).

Results

A total of 60 lipophilic compounds were identified in the preanal gland secretions of male *A. atricollis* (Table I). The main components were 34 steroids (66.9% of TIC), eight fatty acids ranged between *n*-C₁₂ and *n*-C₁₈ (17.7%), six ketones ranged between *n*-C₁₇ and *n*-C₂₅ (6.4%), two forms of tocopherol (2.8%), six waxy esters (2.8%), squalene (2.6%), and three furanones (0.5%). Major compounds were detected in all individuals, although relative proportions of some compounds showed interindividual variability. On average, the two most abundant compounds were cholesterol (14.4% of TIC) and hexadecanoic acid (10.7%), followed by unidentified derivatives of cholest-3-ene (7.7%) and cholest-4-en-3-one (6.1%), respectively.

Table I. Lipophilic compounds found in preanal gland secretions of adult male tree agamas (*Acanthocercus atricol-lis*) ($n = 7$). The relative content of each compound was determined as the percentage of the total ion current (TIC) and is reported as the average ($\pm 1S$). Characteristic ions (m/z) are reported for some unidentified compounds. An asterisk denotes those compounds that were confirmed with authentic standards.

RT ^a [min]	Compound	Content (%) (mean $\pm$ 1S)	RT ^a [min]	Compound	Content (%) (mean $\pm$ 1S)
25.3	Dodecanoic acid	0.12 $\pm$ 0.13*	51.6	Campesterol	0.79 $\pm$ 0.49*
29.1	Tetradecanoic acid	1.10 $\pm$ 0.51*	51.7	Cholest-4-en-3-one	6.14 $\pm$ 2.38*
31.0	Pentadecanoic acid	0.12 $\pm$ 0.10*	52.0	Cholestan-3-one, methyl derivative (?)	0.48 $\pm$ 0.25
31.2	2-Heptadecanone	0.31 $\pm$ 0.24*	52.1	Cholesta-4,6-dien-3-one	0.11 $\pm$ 0.16*
32.4	9-Hexadecenoic acid	0.26 $\pm$ 0.09*	52.2	5,6-Epoxy-cholestan-3-ol	5.75 $\pm$ 2.67
32.7	Hexadecanoic acid	10.71 $\pm$ 2.30*	52.5	Cholestan-3-one, ethyl derivative (?)	0.81 $\pm$ 1.70
34.9	2-Nonadecanone	0.64 $\pm$ 0.34*	52.6	Unidentified steroid (191, 248, 304, 330, 401)	0.49 $\pm$ 0.49
35.1	5-Dodecyldihydro-2(3H)- furanone	0.34 $\pm$ 0.12	52.7	Ergost-8(14)-en-3-ol acetate	0.32 $\pm$ 0.36
35.8	9,12-Octadecadienoic acid	0.14 $\pm$ 0.16*	52.9	Lanost-8-en-3-ol	1.35 $\pm$ 1.32*
35.9	9-Octadecenoic acid	4.73 $\pm$ 2.59*	53.0	Cholest-5-en-3-one	0.52 $\pm$ 0.46*
36.7	Octadecanoic acid	0.72 $\pm$ 1.07*	53.1	Cholestan-3-one, unidentified derivative?	1.22 $\pm$ 0.69
38.4	2-Heneicosanone	1.63 $\pm$ 1.30*	53.3	Stigmast-24(28)-en-3-one	0.19 $\pm$ 0.22*
38.7	Dihydro-5-tetradecyl-2(3H)- furanone	0.10 $\pm$ 0.05	53.7	Cholestane-3,6-dione	4.30 $\pm$ 2.47*
39.9	2-Docosanone	0.18 $\pm$ 0.13*	54.1	Stigmast-4-en-3-one	0.97 $\pm$ 0.45*
41.5	2-Tricosanone	3.26 $\pm$ 2.87*	54.6	Octadecyl hexadecanoate	0.16 $\pm$ 0.16
44.4	2-Pentacosanone	0.41 $\pm$ 0.30*	55.1	Stigmastane-3,6-dione	0.45 $\pm$ 0.41*
45.0	Unidentified furanone	0.11 $\pm$ 0.11	56.3	Octadecyl octadecanoate	0.51 $\pm$ 1.05*
45.7	Squalene	2.62 $\pm$ 1.34*	56.5	Stigmastane-3,6-dione, unidenti- fied derivative?	0.52 $\pm$ 0.60
46.5	Cholest-2-ene	0.09 $\pm$ 0.02*	57.2	Cholest-3-ene, unidentified derivative?	2.80 $\pm$ 2.95
46.9	Cholesta-4,6-dien-3-ol	0.29 $\pm$ 0.09*	57.6	Cholest-3-ene, unidentified derivative?	7.67 $\pm$ 2.72
47.2	Cholesta-3,5-diene	0.46 $\pm$ 0.18*	61.8	Eicosyl hexadecanoate	0.50 $\pm$ 0.64*
47.6	Unidentified steroid (197, 251, 350, 365)	0.07 $\pm$ 0.07	63.1	Cholest-3-ene, unidentified derivative?	3.16 $\pm$ 6.06
48.7	γ -Tocopherol	0.05 $\pm$ 0.06*	65.5	Cholest-3-ene, unidentified derivative?	0.27 $\pm$ 0.48
49.8	Cholestan-3-ol, unidentified derivative?	1.84 $\pm$ 1.44	66.5	Hexadecanoic acid 1,5-pentanediy ester	0.40 $\pm$ 0.29
50.1	D- α -Tocopherol	2.78 $\pm$ 2.78*	66.8	Octadecenyl hexadecanoate	0.52 $\pm$ 1.17*
50.2	Cholesterol	14.40 $\pm$ 8.83*	67.3	Cholest-3-ene, unidentified derivative	0.73 $\pm$ 1.07
50.4	Cholestan-3-ol	2.28 $\pm$ 1.92*	67.8	Octadecyl octadecanoate	0.66 $\pm$ 1.43*
50.5	Cholest-8(14)-en-3-ol	2.01 $\pm$ 1.35*			
50.7	Cholestan-3-one	3.83 $\pm$ 1.97*			
50.8	Cholest-7-en-3-one	0.75 $\pm$ 0.58*			
51.2	Unidentified steroid (233, 274, 382, 400, 415)	0.56 $\pm$ 0.57			
51.3	Unidentified steroid (175, 248, 356, 398)	0.86 $\pm$ 0.66			
51.4	Unidentified steroid (229, 346, 382, 397)	0.41 $\pm$ 0.40			

^a RT, retention time.

Discussion

Preanal secretions of tree agama lizards contain a very high portion of steroids and fatty acids, with cholesterol and hexadecanoic acid being the most abundant compounds. Similar types of compounds have been found in secretions of most of other lizard species examined (Weldon *et al.*, 2008). Cholesterol is often found in femoral

gland secretions of most lizards, but the presence and relative abundance of other steroids seems to be a characteristic of each species. In tree agamas the abundance of cholestan-3-one and cholestan-3-ene is notorious, and, presumably, some of their derivatives, which remained unidentified but seemed similar to those found in European “green” lizards of the genus *Lacerta* (Kopena *et al.*, 2009; Martín and López, 2010b). These ster-

oids may result from the metabolism of cholesterol, but their identification, origin, and potential role in secretions remain to be investigated.

Similarly, hexadecanoic acid, followed by octadecenoic acid, are the most abundant free fatty acids in secretions of this and other lizards (Weldon *et al.*, 2008). However, in contrast to other lizards from arid regions, there were no fatty acids of higher molecular weight, which could be more stable in scent marks (*e.g.* López and Martín, 2005b). This suggests that the substrates usually scent marked by tree agamas (*i.e.* tree trunks) could allow that fatty acids of medium molecular weight persist in scent marks. Also, the large amount of steroids may protect these fatty acids.

The presence of a series of saturated methyl ketones with mostly odd-numbered carbon chains is noteworthy in secretions of tree agamas. A similar bishomologous series of C₁₇–C₂₅ methyl ketones were found in the femoral gland secretions of the phylogenetically unrelated South African sungazer (*Cordylus giganteus*, Cordylidae) (Louw *et al.*, 2007) and in the skin of some snakes (Mason *et al.*, 1990; reviewed in Weldon *et al.*, 2008). These methyl ketones were postulated to arise from free fatty acids that have undergone β -oxidation followed by decarboxylation (Ahern and Downing, 1974). Interestingly, similar ketones from skin glands have a prominent role in the social and sexual behaviour of red garter snakes (Mason *et al.*, 1990), so it would be interesting to test whether ketones might have a similar role in tree agamas.

Waxy esters, together with squalene and tocopherol may function as antioxidants of other compounds in secretions and may contribute to the durability of scent marks on the surface of tree trunks. Finally, we did not find any alcohol in secretions of tree agamas, which coincides with the lack of alcohols in secretions of the few Iguanian lizards species analysed, and contrasts with the frequency and abundance of alcohols in lizards within the Scleroglossa clade (see Weldon *et al.*, 2008 for a review).

Further studies are clearly needed to understand the patterns of presence and abundance of different compounds in femoral or preanal gland secretions of lizards, and how phylogenetic relationships and environmental conditions can explain the characteristics of these secretions. Also, we need to clarify the possible role of the compounds found in femoral gland secretions with potential of being signaling pheromones, such as the series of methyl ketones in secretions of tree agamas, in social organization, and sexual selection processes of lizards.

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