

## Further Analysis of Lipids from the Scent Gland Secretions of Dumeril's Ground Boa (*Acrantophis dumerili* Jan)

John T. Simpson\*, Thomas R. Sharp\*\*

Department of Chemistry, Texas A&M University, College Station, Texas 77843, U.S.A.

William F. Wood

Department of Chemistry, Humboldt State University, Arcata, California 95521, U.S.A.

and

Paul J. Weldon

Department of Biology, Texas A&M University, College Station, Texas 77843, U.S.A.

Z. Naturforsch. **48c**, 953–955 (1993); received April 27/August 18, 1993

Ground Boa, *Acrantophis dumerili*, Scent Gland Lipids, TLC, GC/MS

Cholesterol, cholestadiene, squalene, phenol, C<sub>3</sub>–C<sub>26</sub> fatty acids, and C<sub>16</sub>–C<sub>22</sub> amides were identified from the scent gland secretions of Dumeril's ground boa (*Acrantophis dumerili*). Three amines and a diterpene also were indicated, but the structures of these compounds were not determined.

### Introduction

Analyses of the scent gland secretions of snakes by thin-layer chromatography (TLC) indicate free fatty acids, sterols, and other compounds [1–4]. More detailed analyses, primarily by gas chromatography-mass spectrometry (GC/MS), reveal cholesterol and C<sub>12</sub>–C<sub>25</sub> fatty acids among the scent gland lipids of various snakes [2–8]. Amides [7] and glycerol monoethers [8] also have been reported in the secretions of the western diamond-back rattlesnake (*Crotalus atrox*, Viperidae).

A previous analysis of the scent gland secretions of Dumeril's ground boa (*Acrantophis dumerili*, Boidae) indicated cholesterol, 5-cholesten-3-one, and 2-hydroxypropanoic (lactic), hexadecanoic,

9-octadecenoic, and octadecanoic acids as the main components [5]. We report here the results of further analyses by GC/MS and TLC of lipids in the scent gland secretions of this species.

### Materials and Methods

Scent gland secretions were obtained from adult *A. dumerili* by wiping the cloacal area with a paper towel, pressing the base of the tail, and allowing the glandular exudates to flow into glass vials. Samples were pooled according to sex. Samples used for lipid weight determinations were collected in vials without solvent. Ethyl ether was added to samples used for analysis of volatile components. Chloroform was added to samples for total lipid extraction and acid-base-neutral partitioning. The vials were transported on dry ice and stored at –90 °C.

Total lipids were extracted from the scent gland secretions of *A. dumerili* as described previously [5, 9]. Additional fractionation was done by acid-base-neutral partitioning using 4 N H<sub>3</sub>PO<sub>4</sub> and 4 M NaOH for pH adjustment and extracting with CHCl<sub>3</sub>. Extracts were concentrated by rotoevaporation.

Analytical and preparative TLC using glass plates coated with silica gel HF<sub>254</sub> (Merck, Darmstadt, F.R.G.) also was performed on secretion extracts. TLC plates were developed in benzene:hexane:acetic acid (50:50:2; v/v/v) and then in hexane:ethyl ether:acetic acid (80:20:2; v/v/v). Analytical plates were sprayed with 50% sulfuric acid in methanol or 5% phosphomolybdic acid in ethanol and heated to visualize the lipids. Lipids on the preparative plates were recovered by scraping zones corresponding to the following R<sub>f</sub> ranges: 0–0.13 (zone 1), 0.13–0.25 (zone 2), 0.25–0.50 (zone 3), 0.50–0.67 (zone 4), and 0.67–1 (zone 5). Zone 1 was eluted with CH<sub>3</sub>OH and then with ethyl ether; the other zones were eluted with ethyl ether.

Trimethylsilyl derivatives of lipids were obtained by placing secretion residues in Reacti-vials (Pierce Chemical Co., Rockford, Ill.) and adding TRI-SIL (Pierce Chemical Co.) silylating reagent. The total fatty acid content of the secretions was determined by hydrolysis followed by methylation using 14% BF<sub>3</sub> in methanol. Methyl esters were extracted with hexane. Fatty acids in the acid frac-

\* Present address: NIH-NIMH, Section on Analytical Biochemistry, 9000 Rockville Pike, Bldg. 10 Rm. 3D-40, Bethesda, Maryland 20892, U.S.A.

\*\* Present address: Analytical Research and Development, Pfizer Central Research, Eastern Point Road, Groton, Connecticut 06340, U.S.A.

Reprint requests to J. T. Simpson.

Verlag der Zeitschrift für Naturforschung,  
D-72072 Tübingen  
0939–5075/93/1100–0953 \$ 01.30/0

tion and those eluted from TLC plates were esterified by reaction with diazomethane in ethyl ether [10].

Scent gland lipids subjected to GC/MS were identified by comparison of retention times and/or mass spectra with those of authentic standards, or by derivatization and identification of reaction products. GC/MS analysis of volatile scent gland components was performed on a 5970 Hewlett-Packard mass selective detector interfaced with an HP 5890 gas chromatograph. Separations were performed with a HP-1 cross-linked methyl silicone capillary column (12 m  $\times$  0.2 mm i.d., 0.33  $\mu$  film thickness). The oven temperature was 40 °C for the first 4 min and then increased at a rate of 30 °C/min to a final temperature of 250 °C. The ionization mode was electron ionization at 70 eV, ion source temperature 200 °C, transfer line 260 °C, and scan sample time 0.69 sec over a mass range of  $m/z = 35-350$ . Mass calibration and mass spectrometer operating parameters were established with a perfluorotributylamine standard.

All other GC/MS analyses were performed on a HP 5995C interfaced with a HP 5890A gas chromatograph. Separations were performed on a DB-1701 fused silica bonded stationary phase capillary column (30 m  $\times$  0.32 mm i.d., 0.25  $\mu$  film thickness). A typical temperature program was: 100–300 °C, 10 °C/min, holding at 300 °C for 10 min. The ionization mode was electron ionization at 70 eV, ion source temperature 200 °C, transfer line 300 °C, and scan cycle time 0.71 sec over a mass range  $m/z$  20–620.

## Results and Discussion

Lipids comprise from 3–5% of the total scent gland secretions of *A. dumerili*, as indicated by weights of the extract residues. TLC chromatograms of total lipid extracts indicate prominent bands consistent with sterols and free fatty acids in zones 2 and 3, respectively. A band migrating in zone 5 is more prominent in the TLC profiles of females than in those of males; this was the only evidence for sex differences in scent gland components.

Cholesterol is the most abundant lipid in the secretions of *A. dumerili*, accounting for more than 30% of the total ion current (TIC). This com-

pound has previously been identified by MS in the scent gland secretions of several snakes [2, 5–8].

5-Cholesten-3-one was reported in a previous analysis of the scent gland lipids of *A. dumerili* [5]. However, we failed to detect this compound among either the silylated products of the total lipids or the TLC eluants of this species' secretions. Cholestenone reported in the previous study of *A. dumerili* may have arisen in the GC injection port or on the column from a portion of cholesterol in the secretions that failed to react with the silylating agent.

Propanoic, 2-methylpropanoic, butanoic, 2-methylbutanoic, and 3-methylbutanoic acids and phenol were identified among the volatile components of the secretions; these compounds have not previously been indicated in snake scent gland secretions. Fatty acids (relative percentages in parentheses) detected as methyl esters in the total fatty acid and the acid-partitioned fractions include phenylacetic ( $\leq 1\%$ ), 3-phenylpropanoic ( $\leq 1\%$ ), hexadecanoic (14%), heptadecanoic (2%), 9-octadecenoic (3%), octadecanoic (17%), eicosanoic (8%), docosanoic (9%), tetracosanoic (7%), and hexacosanoic acids (6%). 2-Hydroxypropanoic acid, which had previously been reported in the scent gland secretions of *A. dumerili* [5], is also indicated in our analysis.

A homologous series of even-carbon  $C_{16}-C_{22}$  *n*-amides, primarily hexadecanamide and octadecanamide, was detected among the minor components of the total lipids and as eluants of TLC zone 1. These compounds are indicated by prominent ions in the mass spectra at  $m/z$  59 and  $m/z$  72, products of a McLafferty rearrangement and a  $\beta$ -cleavage, respectively. Minor amounts of  $C_{16}-C_{22}$  amides also have been reported in the scent gland secretions of the western diamondback rattlesnake (*Crotalus atrox*), a viperid [7].

GC/MS analysis of the base fraction of *A. dumerili* secretions indicates three peaks tentatively identified as amines. The mass spectrum of each compound displays a prominent  $m/z$  30 ion (Fig. 1). Selected ion chromatograms indicate  $m/z$  172, 186, and 200 as potential molecular ions for the respective GC peaks. These values are consistent with diamine compounds, however, authentic 1,8-, 1,10-, and 1,12-*n*-diamines matched neither the retention times nor mass spectra of the unknowns. Each mass spectrum contains a  $[M-15]^+$

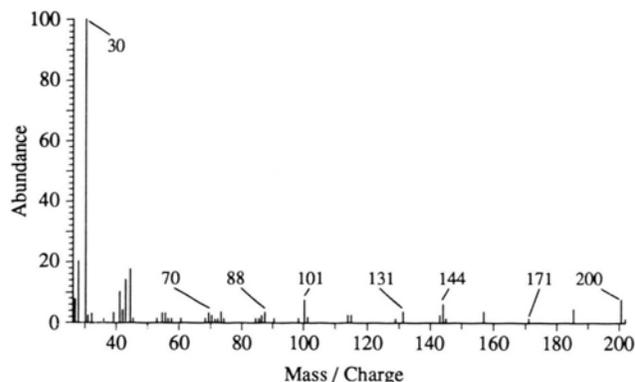


Fig. 1. Mass spectrum of one of three unknown amines detected in the base fraction of *A. dumerili* scent gland secretions.

ion, which is consistent with the loss of a methyl group, and a  $[M-43]^+$  ion, which is consistent with the loss of  $C_3H_7$ . The last two peaks to elute contain a  $[M-29]^+$  ion, which suggests the loss of  $C_2H_5$ . Further studies are needed to characterize these components.

Analyses by GC/MS of TLC zone 5 indicate cholestadiene, squalene, and an unknown isoprenoid eluting just before squalene in the TIC. The cholestadiene exhibits a molecular ion at  $m/z$  368 and the same retention time as cholesta-3,5-diene, a common isomer of this compound. However, a cluster of peaks in the  $m/z$  247 region of the spectrum of authentic cholesta-3,5-diene is not observed in that of the snake-derived compound, indicating that the latter is not the 3,5 isomer. The isoprenoid detected in the secretions is indicated by a prominent ion of  $m/z = 69$  in its mass spec-

trum and by its  $R_f$  value. This compound migrates on TLC plates to the same region as squalene and may be a partially hydrogenated isomer of squalene.

#### Acknowledgements

K. H. Dahm made laboratory space available for this study. M. J. Tanner provided technical assistance. C. M. Garrett, J. B. Murphy (Dallas Zoo, Texas), D. Blody, R. Hudson (Fort Worth Zoological Park, Texas), P. Freed, and K. Peterson (Houston Zoological Gardens, Texas) allowed access to snakes in their care. R. T. Mason and J. S. Waterhouse commented on the manuscript. This study was supported by grants from the Whitehall Foundation, Texas A&M University, and the National Science Foundation (CHE-8705697).

[1] P. D. Oldak, *Copeia* **1976**, 320 (1976).

[2] P. J. Weldon, H. W. Sampson, L. Wong, H. A. Lloyd, *J. Herpetol.* **25**, 367 (1991).

[3] P. J. Tolson, *Occ. Pap. Mus. Zool., Univ. Michigan* **715**, 1 (1987).

[4] M. S. Blum, J. B. Byrd, J. R. Travis, J. F. Watkins II, F. R. Gehlbach, *Comp. Biochem. Physiol.* **38B**, 103 (1971).

[5] J. T. Simpson, P. J. Weldon, T. R. Sharp, *Z. Naturforsch.* **43c**, 914 (1988).

[6] R. R. Razakov, A. S. Sadykov, *Khim. Prir. Soedinenii* **4**, 421 (1986).

[7] P. J. Weldon, R. Oritz, T. R. Sharp, pp. 309-319, in: *Biology of the Pitvipers* (J. A. Campbell, E. D. Brodie, Jr., eds.), Selva Press, Tyler, Tex.

[8] P. J. Weldon, H. A. Lloyd, M. S. Blum, *Experientia* **46**, 774 (1990).

[9] E. G. Bligh, W. J. Dyer, *Can. J. Biochem. Physiol.* **31**, 911 (1959).

[10] D. P. Schwartz, R. S. Bright, *Anal. Biochem.* **61**, 271 (1974).