Identification of Major Lipids from the Scent Gland Secretions of Dumeril's Ground Boa (*Acrantophis dumerili* Jan) by Gas Chromatography-Mass Spectrometry

John T. Simpson

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

Paul J. Weldon

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

Thomas R. Sharp

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

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Ground Boa, *Acrantophis dumerili,* Scent Gland Secretions, GC-MS

The scent gland secretions of Dumeril's ground boa *(Acrantophis dumerili),* pooled from two adult males and a female, were analyzed by gas chromatography-mass spectrometry. 2-Hydroxypropanoic acid, hexadecanoic acid, *cis*-9-octadecenoic acid, octadecanoic acid, cholesterol, and 5-cholesten-3-one were indicated. These results are compared with those obtained in analyses of the scent gland secretions of other snakes.

Introduction

All snakes possess paired scent glands, also called cloacal or postanal glands, that release secretions through two openings at the posteriolateral margin of the cloaca [1, 2]. The malodorous substances produced in these glands typically are discharged, along with feces, when snakes are disturbed. A variety of functions and effects have been attributed to scent gland secretions, including sexual attraction, alarm signalling, and predator repulsion [1].

The components of scent gland secretions are described in several studies, most of which provide information on only the classes of chemicals present. Histochemical tests indicate glyco- or mucoproteins and acidic mucopolysaccharides [1, 2]. Electrophoretic analysis indicates taxonomic differences in scent gland proteins [3]. Scent gland lipids of three families of snakes, examined by thin-layer chromatography (TLC), exhibit variation among species and, in some cases, age classes and sexes [4]. In separate studies, gas chromatography [5] and mass spectrometry [6] have been used to identify free fatty acids in scent gland secretions.

We report here on the identification of organic solvent-extractable compounds from scent gland secretions of Dumeril's ground boa (*Acrantophis dumerili;* Boidae). This is the first detailed description of the scent gland secretion components from this species and family. A previous study of the rubber boa *(Charina bottae bottae)* reported only the lipid classes present [4].

Reprint requests to J. T. Simpson.

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Materials and Methods

Animals

Secretions were obtained from adult snakes (5 and 10 years old), born in captivity at the Fort Worth Zoological Park, Tex., and maintained at the Dallas Zoological Garden, Tex.

Chemicals

HPLC-grade methanol (CH_3OH) and chloroform ($CHCl_3$) were used. TRI-SIL silylating agent was obtained from Pierce, Rockford, Ill. Fatty acid standards were obtained from Alltech Associates, Houston, Tex. Cholesterol was obtained from Sigma Chemical Company, St. Louis, Mo., and 5-cholesten-3-one was obtained from Aldrich Chemical Company, Milwaukee, Wis.

Collection and extraction

Secretions were obtained by first wiping the cloaca with a paper towel, then manually pressing the base of the tail and collecting the exudates in Teflon-lined screw cap vials. A small aliquot of $CHCl_3$ was added

to each vial. The samples were stored at −60 °C. 3 g of pooled secretions from two males and one female were dissolved in 8 ml of $CHCl_3$:CH_3OH:H_2O (1:2:0.8, v/v) and filtered. The filtrate was transferred to a separatory funnel, and 10 ml each of $CHCl_3$ and H_2O were added. The mixture was shaken and allowed to separate. The $CHCl_3$ layer was removed and the aqueous layer was extracted twice with 10 ml of $CHCl_3$. The combined $CHCl_3$ layers were then washed with saturated NaCl and stored overnight over Na_2SO_4 at −10 °C. the $CHCl_3$ extract was filtered and reduced in volume by rotoevaporation at 30 °C to approximately 5 ml. The extract was then transferred to a Teflon-lined screw cap vial and the remainder of the solvent removed under N_2. The weight of the brownish-orange, malodorous residue was 116.9 mg (*ca.* 4% by weight of the original total secretion). The residue was redissolved in 8 ml of $CHCl_3$ and refrigerated at 4 °C.

Derivatization

An aliquot of the $CHCl_3$ extract containing 1 mg of material was placed in a septum-capped Reactivial (Pierce, Rockford, Ill.) and the solvent was removed under N_2. A 100 µl aliquot of TRI-SIL silylating reagent was added to the residue, the vial shaken for 5 min and left at room temperature for 15 min, followed by injection of 1 µl into the gas chromatograph-mass spectrometer (GC-MS).

Gas chromatography-mass spectrometry

GC-MS analysis was done on a VG Analytical 70S high resolution double focusing magnetic sector mass spectrometer, with attached VG Analytical 11/250J datasystem, interfaced with a Hewlett-Packard 5890A gas chromatograph through a direct inlet line. Ionization mode was electron impact at 70 eV, ion source temperature 210 °C, resolving power 2500, transfer lines 300 °C, and scan cycle time 1 sec over the mass range *m/z* 20–620. Mass spectrometer operating parameters were optimized and mass calibration established using a perfluorokerosene standard (PCR, Gainesville, Fla.). GC separations were performed on a fused silica capillary column coated with a DB-1 bonded stationary phase (30 m × 0.32 mm i.d., 0.25 µ film thickness, J&W Scientific, Folsom, Cal.) using a typical temperature program; 100–300 °C @ 10 °C/min, holding at 300 °C for 10 min. Total elution time for underivatized and TRI-SIL treated materials was 25 min.

Results

The reconstructed total ion current chromatogram (TIC) from analysis of an aliquot of underivatized extract is shown in Fig. 1. Components were identified by three criteria: comparison of retention times of secretion components and authentic standards, comparison of mass spectra of standards and secretion components, trimethylsilylation and identification of the corresponding derivatives (Fig. 2). Mass spectral matching was made using both visual comparison and computer-based comparison *via* searching against the on-line version of the National Bureau of Standards [7] mass spectral database available on the GC-MS datasystem.

Five major components were identified: hexadecanoic (palmitic) acid, *cis*-9-octadecenoic (oleic) acid,

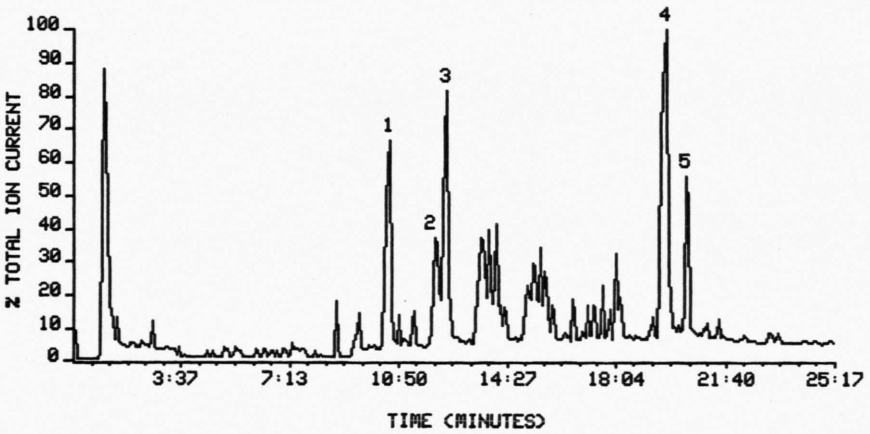

Fig. 1. Gas chromatogram of underivatized $CHCl_3$ extract. Peak: 1, hexadecanoic acid; 2, *cis*-9-octadecenoic acid; 3, octadecanoic acid; 4, cholesterol; 5, 5-cholesten-3-one.

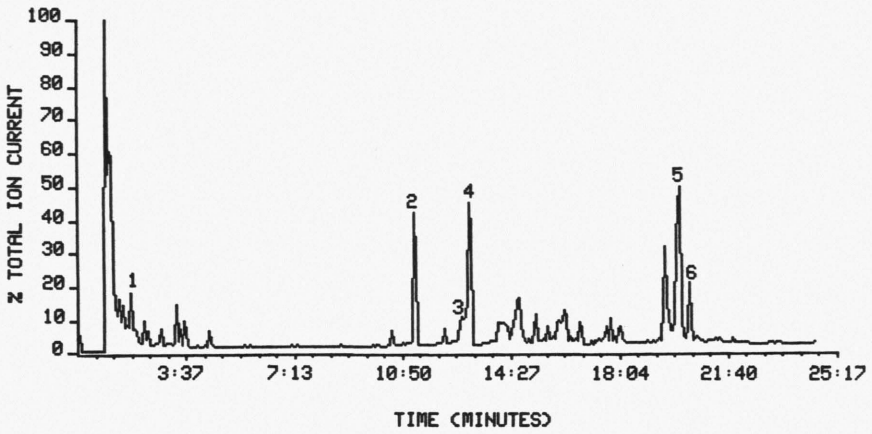

Fig. 2. Gas chromatogram of trimethylsilyl (TMS) derivatized $CHCl_3$ extract. Peak: 1, TMS 2-trimethylsilyloxypropanoate; 2, TMS hexadecanoate; 3, TMS *cis*-9-octadecenoate; 4, TMS octadecanoate; 5, cholesterol TMS ether; 6, 5-cholesten-3-one.

octadecanoic (stearic) acid, cholesterol, and 5-cholesten-3-one (cholestenone). Stereochemical and double-bond position designations for the octadecenoic acid and cholestenone are assumed here based upon comparison of standards with unknowns. Experiments have not been performed to verify these assignments.

Octadecenoic acid was sufficiently separated from octadecanoic acid to permit identification. Groups of chromatographically unresolved components eluting between octadecanoic acid and cholesterol occur at positions consistent with C_{20}, C_{22} and (possibly) C_{24} fatty acids. Examination of the mass spectra recorded in these regions support this suggestion, but confirmation must await study on a more selective column.

Cholesterol and 5-cholesten-3-one can be clearly identified in the region where steroidal compounds elute. Trimethylsilylation converts cholesterol to its trimethylsilyl (TMS) ether, while cholestenone, being unreactive to derivatization under the conditions used, remains unchanged. Other steroids, eluting just prior to cholesterol, are also indicated. However, sufficient chromatographic resolution was not obtained for conclusive identification.

A small amount of 2-hydroxypropanoic (lactic) acid, which was not apparent in the chromatogram of the underivatized material, was identified by characteristic mass spectral fragmentation as its *bis*-TMS derivative (Fig. 2) [8].

Discussion

Previous studies indicate that scent gland secretions of snakes are complex mixtures of a variety of compound classes. Most attention has been given to the lipids [1, 4]. The thin-layer chromatograms of boid, colubrid, and viperid scent gland secretions displayed bands consistent with sterols, sterol esters, and free fatty acids. Many exhibited a band consistent with triglycerides [4].

Analysis of scent gland secretions of the Texas blind snake (*Leptotyphlops dulcis;* Leptotyphlopidae) indicated a glycoprotein and fatty acids [5]. GC retention times were used to identify the free fatty acids. Sterols were indicated in a TLC profile, but they were not investigated further.

The scent gland secretions of three viperids, the common adder *(Vipera berus),* the saw-scaled viper *(Echis carinatus),* and the mamushi *(Agkistrodon halys),* have also been investigated [6]. Crude secretions, found to contain free fatty acids and cholesterol, were analyzed by direct tandem mass spectrometric and other techniques, without benefit of any solvent or chromatographic fractionation [6].

Separation, as well as structural information of the various chemical components in scent gland secretions, can be obtained by the use of GC-MS. The mass spectral data, coupled with the retention time shifts of derivatives, provides sufficient evidence to assign structures to the various components. Using this methodology, we found that the chloroform-soluble scent gland compounds of *A. dumerili* contained 2-hydroxypropanoic acid, hexadecanoic acid, octadecanoic acid, octadecenoic acid, cholesterol and cholestenone. The identification of cholestenone represents the first documentation of a steroid other than cholesterol from a reptile skin gland. We also found at least two other as yet unidentified steroids, in lower concentrations.

Investigations of free fatty acids in leptotyphlopid [5], viperid [6], and, from our analysis, boid scent gland exudates have revealed fatty acids ranging in carbon chain length from C_{12} to C_{22}. Some differences, possibly reflecting taxonomic variation, are suggested. An analysis of viperid secretions, for example, indicated C_{15} to C_{18} compounds in two viperines, the saw-scaled viper *(Echis carinatus)* and the common adder *(Vipera berus)*, but the mamushi *(Agkistrodon halys)*, a crotaline, displayed only C_{20} to C_{22} acids [6]. Blum *et al.* [5] found primarily C_{16}, $C_{18:1}$ and $C_{18:2}$ acids in their analysis of the Texas blind snake *(Leptotyphlops dulcis)*. The unsaturated C_{18} acids accounted for 67% of the free fatty acids identified. The scent glands of *A. dumerili* contain 2-hydroxypropanoic acid, which has not been reported previously in scent gland secretions. Determination of whether taxonomic differences exist in the composition of these secretions will require studies involving larger sample sizes of each species investigated. Sex and developmental differences, possible sources of variation in scent gland secretions [3, 6], also need to be considered.

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