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## Lipids in the Gular Gland Secretion of the American Alligator (Alligator mississippiensis)

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Lipids from the gular glands of the American alligator (Alligator mississippiensis) were examined by gas chromatography-mass spectroscopy.  $C_{14}$ ,  $C_{16}$ , and  $C_{18}$  fatty acids, squalene, and  $\alpha$ -tocopherol (vitamin E) were detected in nearly all samples. Cholesterol was detected clearly in several samples.

## Introduction

All extant crocodilians possess two large integumental glands, referred to by Neill [1] as gular glands, on the ventral surface of the lower jaw adjacent to each mandibular ramus. Some authors suggest that these organs produce pheromones, an idea critically discussed by Neill [1]. Olfactometric tests indicate that yearling American alligators (Alligator mississippiensis) detect airborne gular gland secretions from adult male conspecifics [2], but no pheromones have been demonstrated.

Thin-layer chromatography (TLC) of the gular gland lipids of *A. mississippiensis* indicates bands consistent with ceramides, sterols, free fatty acids and other lipid classes, and a carbon-13 nuclear magnetic resonance (CMR) spectrum of whole gular gland secretion displays signals consistent with some of these compounds [3]. The detailed structures of gular gland chemicals, however, have not been determined for any crocodilian.

We report here the structure determination of chemicals from the gular glands of *A. mississippiensis*.

## **Materials and Methods**

Gular gland secretions were obtained from nine immature (total lengths = 98-118 cm) and eight adult alligators (1.5-3.0 m) from Port Arthur, Texas

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(Murphree Wildlife Management Area) and 21 adults (1.6–3.3 m) from Grand Chenier, Louisiana (Rockefeller Wildlife Center) during September. Secretions from immature (unsexed) and adult alligators were kept separate. The adults' secretions were pooled, with up to eight individuals per collecting vial; materials were pooled according to sex for six males and three females from Louisiana. The adult alligators were sampled within one hour after sacrifice. Samples from living immature alligators were taken after they had been hand-captured and confined with 15 or more individuals for several hours.

Secretions were collected by manually everting and squeezing each gland while a glass capillary tube was inserted into the single duct opening. The tube was withdrawn and the end packed with secretion was broken off into a glass vial containing 3 ml of methylene chloride  $(CH_2Cl_2)$ . The secretions were shipped on dry ice and kept frozen  $(-4 \, ^{\circ}C)$  before analysis.

The capillary tubes were crushed with a glass rod and the  $CH_2Cl_2$  extracts were drawn off with pipettes and analyzed by capillary gas chromatography-mass spectroscopy (GC-MS), using either an SE-54 (30 m $\times$ 0.32 m $\mu$ , 0.25  $\mu$ m) or SPB-5 (30 m $\times$ 0.32 m $\mu$ , 0.25  $\mu$ m) column connected to a Finnigan 4500B mass spectrometer. Spectra and retention times were compared to authentic, commercially available compounds.

## **Results and Discussion**

Sterols are widely documented in vertebrate skin and skin gland secretions, including lizard and snake shed skin extracts [4–6], snake scent gland secretions [7], tortoise mental gland exudates [8], and crocodilian paracloacal gland secretions [9, 10]. The chemical structures of these sterols have not been resolved in most of these studies, which generally rely solely upon TLC migration of bands as a means of characterization. A TLC band consistent with sterols was indicated in *A. mississippiensis* gular gland secretion, but a CMR spectrum of this material failed to display all absorptions expected for cholesterol [3]. Our GC-MS analysis indicates trace or minor amounts of cholesterol in the gular gland secretion (Table I).

Squalene, a precursor of cholesterol, occurs in the skin gland exudates of a variety of mammals [11]. A



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Table I. Compounds isolated in major (maj $> 30\%$ ), minor (min $< 20\%$ ), or
trace (tr $< 5\%$ ) amounts from alligator gular glands. The number of individuals
and, for adults, sexes contributing to each pooled sample are indicated.

Compound mw	Louis 6♂	siana 3♀	3♂/1♀	6♂/2♀	Texas 7♂/1♀	immature 9
tetradecanoic 228 acid	min	min	min	tr	tr	-
hexadecanoic 256 acid	min	maj	maj	maj	maj	maj
octadecanoic 284 acid	min	min	min	min	maj	min
cholesterol 386	tr	min	?	?	?	tr
squalene 410	maj	maj	min	maj	tr	maj
α-tocopherol 430	tr	min	min	tr	tr	tr

TLC survey of snake shed skin extracts failed to indicate squalene [5], and this compound has not heretofore been indicated in reptile skin gland secretions. Squalene was detected in high concentrations in most alligator gular gland samples we examined.

The  $C_{14}$ ,  $C_{16}$ , and  $C_{18}$  free fatty acids indicated in gular glands are typical of those that occur in a wide variety of vertebrate skin and skin gland secretions [11], including the exudate of tortoise mental glands [8], paired skin glands on the lower jaw of some chelonians not homologous with crocodilian gular glands.

 $\alpha$ -Tocopherol (vitamin E) was detected in trace or minor amounts in all gular gland samples examined. This compound also has been indicated in the secretion of the paracloacal glands of A. mississippiensis [10]. Weldon et al. [10] suggest that this substance is delivered to the paracloacal glands via a circulatory or lymphatic route. The extensive network of capillaries in the capsule and septa of the gular glands of A. mississippiensis [3] may transport  $\alpha$ -tocopherol to the gular gland secretion.

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