Age-Class Differences in Lipids from the Paracloacal Glands of the American Alligator (*Alligator mississippiensis*)

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The paracloacal gland secretions of immature and adult American alligators (*Alligator mississippiensis*) from Louisiana were analyzed by gas chromatography/mass spectrometry and nuclear magnetic resonance spectroscopy to investigate age-class differences in lipid composition. The secretions of both immature and adult alligators contain saturated C_{14} and C_{16} free fatty acids and decyl, undecyl, dodecyl, tetradecyl, and hexadecyl acetates. Compounds observed only in immature alligators include dodecanoic acid, C_{12} and C_{14} alcohols, C_{10}–C_{14} butanoates, an array of C_8–C_{18} 3-methylbutanoates, C_{12}–C_{15} hexanoates, C_{12} and C_{14} octanoates, a C_{12} decanoate, C_{10}–C_{14} dodecanoates, C_{10}–C_{16} tetradecanoates, C_{12} and C_{14} hexadecanoates, a C_{12} octadecanoate, and a diterpene hydrocarbon identified as (*E,E*)-7,11,15-trimethyl-3-methylenehexadeca-1,6,10,14-tetraene (β-springene). Compounds observed only in adults include several C_7–C_{16} free fatty acids and certain C_{10}–C_{18} acetates. The age-class differences we observe in the paracloacal gland lipids of alligators from Louisiana are similar to those previously reported for alligators from Texas.

Introduction

All extant crocodilians possess paired paracloacal glands that open through ducts near the vent. These skin glands generally are thought to produce pheromones used to attract mates and/or mark nest sites (Weldon and Ferguson, 1993). Analyses by gas chromatography/mass spectrometry (GC/MS) of the paracloacal gland secretions of alligators (*Alligator* spp.) and caimans (*Paleosuchus* spp.) indicate acetates and other esters (Weldon *et al.,* 1988; Shafagati *et al.,* 1989; Dunn *et al.,* 1993), free fatty acids (FFA) and alcohols (Shafagati *et al.,* 1989; Dunn *et al.,* 1993), and hydrocarbons (Avery *et al.,* 1993; Mattern *et al.,* 1997).

Sources of variation in the composition of paracloacal gland secretions have been suggested in several studies. Taxonomic variation was revealed by thin-layer chromatography of the secretion extracts of 21 crocodilians, some of which displayed apparent species- or genus-unique profiles (Weldon and Tanner, 1991). Sex differences were observed by thin-layer chromatography and GC/MS of the secretions of the Chinese alligator (*Alligator sinensis*), only males of which possess a diterpene hydrocarbon and a diterpene ketone recently identified as 4,8,12-trimethyl-1-(1-methylethenyl)-3,6,11-cyclotetradecatriene (cembrene A) and 4,8,12-trimethyl-1-(1-methylethenyl)-3,7-cyclotetradecadien-10-one, respectively (Dunn *et al.,* 1993; Mattern *et al.,* 1997).

Age-class differences in paracloacal gland products also have been observed. A GC/MS analysis of free-ranging *A. mississippiensis* revealed that adults possess esters consisting primarily of C_{12}–C_{18} acetates, while immature alligators possess butanoates, 3-methylbutanoates, hexanoates, and higher molecular weight, straight-chain esters (Weldon *et al.,* 1988). An unidentified diterpene hydrocarbon with a molecular weight of 272 was detected in high concentrations in all samples of immature alligators, but not in those of adults (Shafagati, 1989).

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We report the results of a further comparison of the paracloacal gland lipids of immature and adult *A. mississippiensis*. Our study confirms many of the age-class differences in secretion composition previously reported for this species, and it suggests further differences. In addition, we identify previously unreported compounds from the paracloacal glands of *A. mississippiensis*, including the diterpene hydrocarbon present in immature alligators, which we identify as (*E,E*)-7,11,15-trimethyl-3-methylenehexadeca-1,6,10,14-tetraene (β-springene).

Material and Method

Paracloacal gland secretions were obtained from captive-raised, immature and free-ranging, adult *A. mississippiensis* from Louisiana. Samples of immature alligators (total lengths = 41–91 cm) were obtained at the Rockefeller Wildlife Refuge in Grand Chenier, Louisiana, during late July from 13-month-old subjects hatched from eggs collected in Vermilion parish. Hatchlings were fed Burris 45% Alligator Food (Burris Pet Food, Franklinton, Louisiana), which contains fish meat, dried blood meal, corn gluten meal, hydrolyzed feather meal, and dehulled soybean meal. Samples of adults (total lengths = 1.5–3.0 m) were obtained during early September from freshly hunted alligators from Vermilion parish.

The secretions of (unsexed) immature alligators were collected by manually grasping the base of the tail and compressing the region around the cloacal orifice to compress both glands simultaneously. The light yellow fluids discharged from the everted gland duct openings were taken up into capillary tubes, which were then broken off into glass vials that were then sealed with polyethylene-lined caps. Samples were pooled in three vials, two of which contained secretions from nine individuals each; the third vial contained secretions from 28 individuals.

The secretions of adult alligators were collected by manually palpating and compressing each gland and allowing the orange-yellow exudates to flow into glass vials placed near the gland duct openings. Samples were pooled in six vials containing the following numbers of males and females, respectively: 0/4, 1/2, 2/2, 2/3, 3/1, and 4/0. Several milliliters of CH_2Cl_2 were added to each vial before they were sealed with polyethylene-lined caps and placed on dry ice. The vials were stored at −10 °C.

The crude CH_2Cl_2 extracts were divided into two portions. Diazomethane was added to one portion to form methyl ester derivatives of the FFA present. The other portion was shaken three times with a 10% solution of $NaHCO_3$ to extract the FFA; the remaining secretions were then analyzed for possible native methyl esters.

Analyses were conducted on the following two capillary GC/MS systems: a 30 m×0.32 mm SPB-5 column (0.25 μm) connected to a Finnigan-MAT 4500B mass spectrometer, and a 60 m×0.25 mm DB-1 column (0.25 μm) connected to a Finnigan INCOS XL mass spectrometer with a Data General NV3200 data system. Both spectrometers were operated in the electron impact mode at 70 eV using helium as a carrier gas; the temperature of each was programmed from 50 °C to 300 °C at 5 °C/min.

A diterpene hydrocarbon detected in immature alligators was further investigated by proton (^{1}H NMR) and carbon-13 nuclear magnetic resonance (^{13}C NMR) spectroscopy. This compound was isolated by evaporating CH_2Cl_2 from each sample under a stream of N_2. The bulk crude sample was dissolved in pentane and fractionated on a silicic acid (100–200 mesh) column eluted with pentane. A mixture of ether/pentane (1:9, v/v) was then passed through the column to elute the more polar lipids. Pentane was removed from the first fraction under N_2 and the residue was analyzed by GC/MS.

Part of the first fraction, which contained the unknown hydrocarbon, was dissolved in $CDCl_3$ and analyzed by 2D correlated spectroscopy (COSY) using a General Electric QE 300 MHz spectrometer with tetramethylsilane as an internal standard. The data acquisition parameters were as follows: sample size, 120 mg; 512 data points per FID; total acquisition time, 301.158 min; increment for t_1 evolution, 3 μs; relaxation delay, 2 s; scan count, 64; acquisition point, 1024.

The other part of the first fraction was dissolved in pure anhydrous EtOH and then hydrogenated at 30 psi overnight using Pd/Al_2O_3 on alumina (5%) as the catalyst. The reaction mixture was filtered, the EtOH was removed by rotary evaporation, and the product was analyzed by GC/MS.

Methyl 3,7,11-trimethyldodecanoate was synthesized to compare it with the methyl ester deriv-

ative of 3,7,11-trimethyldodecanoic acid, a suspected component of the secretions. This compound was synthesized by adding 0.5 ml of (*E,E*)-3,7,11-trimethyldodeca-2,6,10-trienol (farnesol) to EtOH (15 ml) and hydrogenating the mixture with Pd/Al_2O_3 at room temperature and 30 psi pressure. The mixture was shaken overnight and then filtered. The EtOH was removed by rotary evaporation. Analysis by GC/MS revealed that farnesol was completely reduced to the corresponding alcohol, 3,7,11-trimethyldodecanol. This product (0.39 g) was dissolved in 12 ml of ether/acetone (5:1, v/v). CrO_3 (1 g) and H_2SO_4 (1 ml) were added with continuous stirring for 12 h at room temperature. The mixture was filtered and dried over Na_2SO_4. Diazomethane was added to a small portion of the product, which was analyzed by GC/MS.

Farnesyl 3-methylbutanoate was synthesized to compare it with a natural component of the secretions. This compound was synthesized by adding a mixture of (*E,E*)-farnesol (2.03 g) and pyridine (0.71 g) to anhydrous ether (10 ml) in a three-necked flask fitted with a reflux condenser with a $CaCl_2$ tube attached and a dropping funnel. The mixture was cooled on ice and 3-methylbutanoyl chloride (1.20 g) was added dropwise through the dropping funnel. The ice bath was removed and the mixture was allowed to stand for 3 h. H_2O (20 ml) was added to the mixture and it was allowed to stand overnight. The mixture was shaken several times with H_2O and then with $NaHCO_3$. The ether layer was dried over Na_2SO_4. The product (1.40 g) was purified by flash chromatography using petroleum ether/pentane (1:9, v/v) as the eluant, and then analyzed by GC/MS. The mass spectrum, which displayed an M^+ ion at 306, was consistent with farnesyl 3-methylbutanoate.

To differentiate between farnesyl 3-methylbutanoate and its positional analogs, the retention times and mass spectra of farnesyl 2-methylbutanoate and farnesyl pentanoate also were compared with the natural product. These compounds also were synthesized from the appropriate acid chlorides.

Results and Discussion

The results of our analysis of the paracloacal gland lipids of *A. mississippiensis* agree with those of a previous GC/MS analysis of this species by indicating an abundance of straight-chain esters (Weldon *et al.,* 1988) (Table I). Identification of the acetates is facilitated by the presence of $CH_3C{=}O^+$ and $CH_3CO_2H_2^+$ peaks in their mass spectra at *m/z* 43 and *m/z* 61, respectively. For example, the acid portions of the C_4, C_5, and C_6 esters were characterized from the $RCO_2H_2^+$ ion fragments resulting in peaks at *m/z* 89, *m/z* 103, and *m/z* 117, respectively. The peaks at *m/z* 71, *m/z* 85, and *m/z* 99 are due to $RC{=}O^+$ ion fragments of the same esters. The alcohol portions of the esters were characterized from the $R'-H^+$ ion fragments resulting in peaks at *m/z* 140 (C_{10}), *m/z* 168 (C_{12}), and *m/z* 196 (C_{14}), where R and R' are corresponding alkyl groups of a saturated molecule.

The secretions of both immature and adult alligators contain decyl, undecyl, dodecyl, tetradecyl, and hexadecyl acetates; dodecyl and hexadecyl acetates were indicated as major components in some samples. Most esters, however, were differentially distributed between the age-classes. An array of C_8-C_{18} 3-methylbutanoates was observed in all samples of immature alligators. These compounds, $C_{10}-C_{14}$ butanoates, and other low molecular weight esters eluted in order of increasing carbon-chain length of the alcohol function, but decreasing chain length of the acid function. For example, the $C_{12}-C_6$, $C_{14}-C_4$, and $C_{16}-C_2$ compounds eluted respectively as separate peaks. Higher molecular weight esters, including $C_{12}-C_{15}$ hexanoates, C_{12} and C_{14} octanoates, a C_{12} decanoate, $C_{10}-C_{14}$ dodecanoates, $C_{10}-C_{16}$ tetradecanoates, C_{12} and C_{14} hexadecanoates, and a C_{12} octadecanoate, were observed only in immature alligators in trace amounts; these esters also were reported in the secretions of free-ranging, immature alligators from Texas (Weldon *et al.,* 1988).

We observed decenyl, undecenyl, dodecenyl, tetradecenyl, pentadecyl, pentadecenyl, hexadecadienyl, heptadecenyl, and several C_{18} acetates in one or more samples of adults. Hexadecenyl acetate and octadecenyl acetate were major components in some samples. Weldon *et al.* (1988) also observed hexadecenyl acetate as a major component of adult *A. mississippiensis.* We detected trace amounts of hexadecyl 3-methylbutanoate in one pooled sample of adults; this was the only 3-methylbutanoate observed in adults.

Table I. Lipids detected in trace (tr, <5%), minor (min, <20%), or major (maj, >25%) quantities in the paracloacal gland secretions of immature and adult *A. mississippiensis*. Percentages are given within each compound class. Compounds are listed within each chemical class in order of increasing molecular weight (m.wt.). The number of individuals contributing secretions to each pooled sample are shown. Compounds reported by Weldon *et. al.* (1988) and Shafagati (1989) in immature ([i]) and adult ([a]) *A. mississippiensis* are indicated.

Compound	m.wt.	Age-class								
		Immature			Adult (males/females)					
		9	9	28	0/4	1/2	2/2	2/3	3/1	4/0
Hydrocarbons										
(*E,E*)-β-Springene[i]	272	maj	maj	maj	–	–	–	–	–	–
Alcohols										
Dodecanol	186	tr	tr	tr	–	–	–	–	–	–
Tetradecanol	214	tr	tr	tr	–	–	–	–	–	–
Fatty Acids*										
Heptanoic acid	130	–	–	–	–	–	–	–	tr	–
Undecenoic acid	184	–	–	–	–	–	–	–	tr	–
Undecanoic acid	186	–	–	–	–	–	–	–	tr	–
Dodecanoic acid	200	tr	tr	tr	–	–	–	–	–	–
Tetradecanoic acid	228	tr	tr	tr	tr	tr	tr	tr	tr	tr
3,7,11-Trimethyldodecanoic acid	242	–	–	–	tr	tr	tr	tr	tr	tr
Pentadecanoic acid	242	–	–	–	–	–	–	tr	tr	–
Hexadecenoic acid	254	–	–	–	–	–	–	tr	tr	–
Hexadecanoic acid[i,a]	256	maj	maj	maj	tr	tr	tr	tr	min	tr
Octadecadienoic acid	280	–	–	–	tr	tr	tr	tr	tr	tr
Octadecenoic acid[i,a]	282	–	–	–	tr	tr	tr	tr	tr	tr
Octadecanoic acid[i,a]	284	–	–	–	tr	tr	tr	tr	tr	tr
Esters										
Decenyl acetate	198	–	–	–	tr	tr	tr	tr	tr	tr
Decyl acetate[i]	200	tr	tr	tr	tr	tr	tr	tr	tr	tr
Undecenyl acetate	212	–	–	–	tr	tr	tr	–	–	–
Octyl 3-methylbutanoate	214	tr	tr	tr	–	–	–	–	–	–
Undecyl acetate	214	tr	tr	tr	tr	tr	tr	–	–	–
Dodecenyl acetate	226	–	–	–	min	min	min	min	tr	tr
Decyl butanoate	228	tr	tr	tr	–	–	–	–	–	–
Dodecyl acetate[i,a]	228	maj	maj	maj	min	min	min	min	tr	tr
Tridecyl acetate	242	tr	tr	tr	–	–	–	–	–	–
Decyl 3-methylbutanoate[i]	242	min	min	min	–	–	–	–	–	–
Tetradecenyl acetate	254	–	–	–	min	min	min	min	min	min
Undecyl 3-methylbutanoate	256	tr	tr	tr	–	–	–	–	–	–
Dodecyl butanoate[i]	256	min	min	min	–	–	–	–	–	–
Tetradecyl acetate[i,a]	256	min	min	min	min	min	min	min	min	min
Farnesyl acetate	264	–	–	–	min	min	tr	tr	tr	tr
Pentadecenyl acetate	268	–	–	–	tr	–	–	–	–	–
Dodecyl 3-methylbutanoate[i]	270	maj	maj	maj	–	–	–	–	–	–
Pentadecyl acetate[a]	270	–	–	–	tr	min	min	tr	tr	tr
Hexadecadienyl acetate[a]	280	–	–	–	min	min	min	min	min	min
Hexadecenyl acetate[a]	282	–	–	–	maj	maj	maj	maj	maj	maj
Dodecyl hexanoate[i]	284	min	min	min	–	–	–	–	–	–
Tetradecyl butanoate	284	min	min	min	–	–	–	–	–	–
Hexadecyl acetate[i,a]	284	min	min	min	maj	maj	maj	maj	maj	maj
Heptadecenyl acetate[a]	296	–	–	–	tr	–	–	tr	–	–
Tetradecyl 3-methylbutanoate[i]	298	min	min	min	–	–	–	–	–	–
Farnesyl 3-methylbutanoate	306	min	min	min	–	–	–	–	–	–
Octadecadienyl acetate[a]	308	–	–	–	min	min	min	min	min	min
Octadecenyl acetate[a]	310	–	–	–	min	maj	min	min	min	min
Dodecyl octanoate	312	tr	tr	tr	–	–	–	–	–	–
Tetradecyl hexanoate	312	tr	tr	tr	–	–	–	–	–	–
Octadecyl acetate[i]	312	–	–	–	tr	tr	tr	tr	tr	tr
Farnesyl hexanoate	320	tr	tr	tr	–	–	–	–	–	–
Hexadecyl 3-methylbutanoate	326	tr	tr	tr	–	–	tr	–	–	–
Decyl dodecanoate	340	tr	tr	tr	–	–	–	–	–	–
Dodecyl decanoate	340	tr	tr	tr	–	–	–	–	–	–
Tetradecyl octanoate	340	tr	tr	tr	–	–	–	–	–	–
Octadecenyl 3-methylbutanoate	352	tr	tr	tr	–	–	–	–	–	–
Octadecyl 3-methylbutanoate	354	tr	tr	tr	–	–	–	–	–	–
Decyl tetradecanoate[i]	368	tr	tr	tr	–	–	–	–	–	–

Table I. (Continued).

Compound	m.wt.	Age-class								
		Immature			Adult (males/females)					
		9	9	28	0/4	1/2	2/2	2/3	3/1	4/0
Dodecyl dodecanoate[i]	368	tr	tr	tr	–	–	–	–	–	–
Tridecyl dodecanoate	382	tr	tr	tr	–	–	–	–	–	–
Dodecyl tetradecanoate[i]	396	tr	tr	tr	–	–	–	–	–	–
Tetradecyl dodecanoate[i]	396	tr	tr	tr	–	–	–	–	–	–
Dodecyl hexadecanoate[i]	424	tr	tr	tr	–	–	–	–	–	–
Tetradecyl tetradecanoate[i]	424	tr	tr	tr	–	–	–	–	–	–
Dodecyl octadecanoate[i]	452	tr	tr	tr	–	–	–	–	–	–
Tetradecyl hexadecanoate[i]	452	tr	tr	tr	–	–	–	–	–	–
Hexadecyl tetradecanoate[i]	452	tr	tr	tr	–	–	–	–	–	–

* Identified as methyl esters.
– Denotes compound was not detected.

Several farnesyl esters, the mass spectra of which display *m/z* values at 204, 161, 136, 121, and 69, were observed in the secretions of immature or adult alligators. Immature alligators possess farnesyl 3-methylbutanoate, with *m/z* values at 306 (M^+) and 85 (Fig. 1), and farnesyl hexanoate, with *m/z* values at 320 (M^+), 117, and 99. Adult alligators possess farnesyl acetate, with *m/z* values at 264 (M^+), 61, and 43. These compounds, which have not previously been reported in paracloacal gland secretions, were confirmed by comparisons of the GC retention times and mass spectra of synthetic compounds, as shown for farnesyl 3-methylbutanoate (Fig. 2).

As in Shafagati's (1989) analysis of *A. mississippiensis* from Texas, all samples of our immature alligators, but none of those of adults, contained high concentrations of a diterpene hydrocarbon exhibiting a molecular weight of 272. The mass spectrum of this compound displayed the following *m/z* values (rel. int.): 272 (M^+) (2), 257 (2), 229 (1), 215 (0.5), 203 (2), 201 (1), 189 (2), 187 (4), 175 (2), 161 (8), 159 (3), 147 (4), 137 (3), 135 (5), 133 (16), 120 (15), 119 (10), 109 (8), 107 (10),

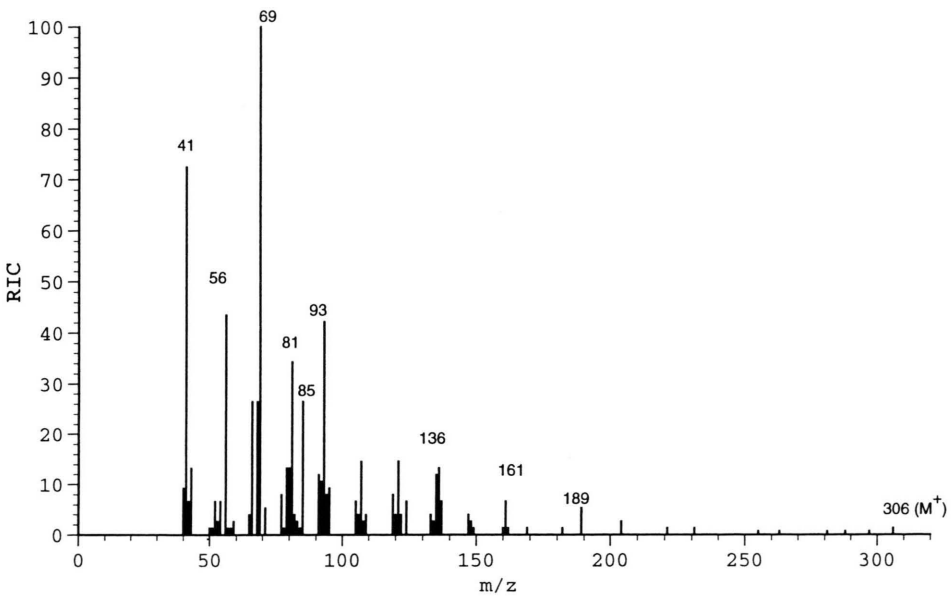

Fig. 1. Mass spectrum of farnesyl 3-methylbutanoate.

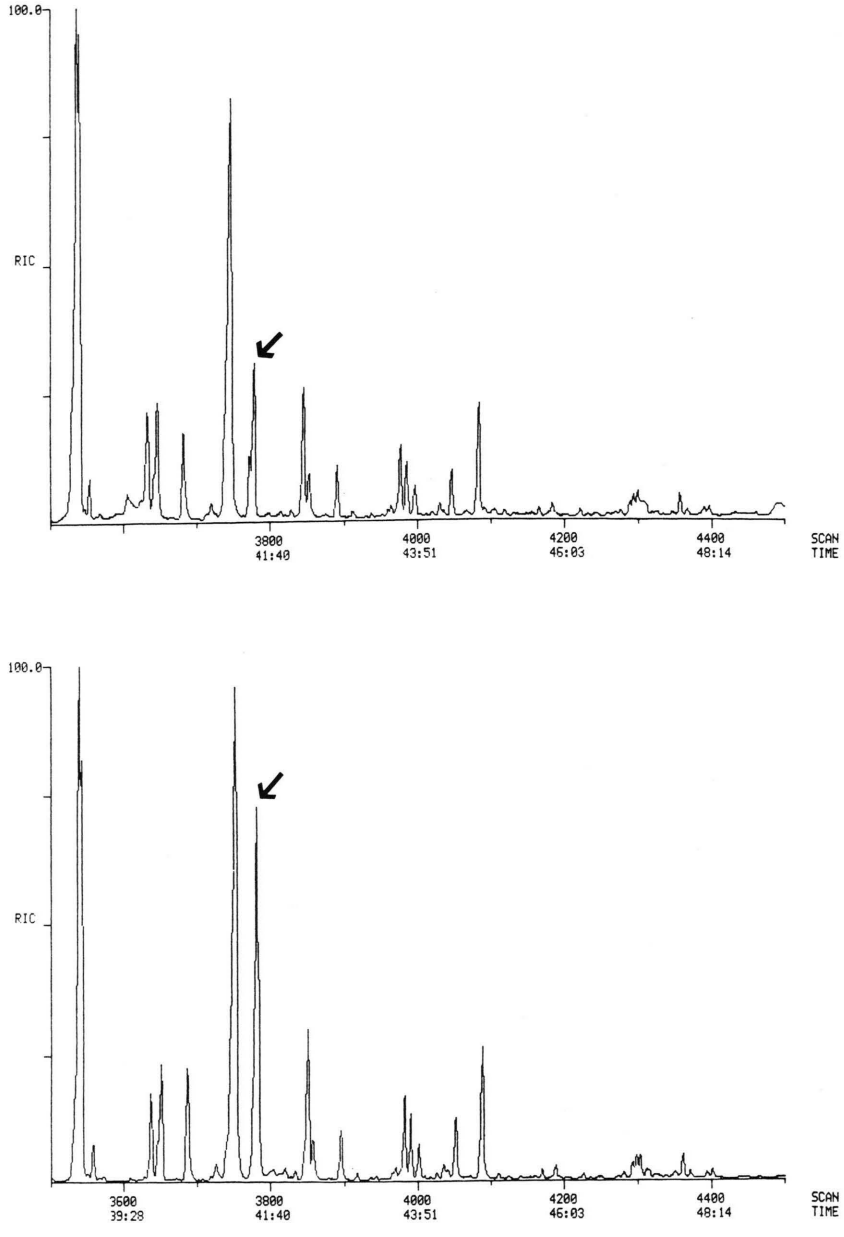

Fig. 2. Gas chromatograms of paracloacal gland secretions of immature *A. mississippiensis*. The top chromatogram shows relative ion current (RIC) of extract with peak of suspected farnesyl 3-methylbutanoate (arrow). The bottom chromatogram shows RIC of extract with added synthetic farnesyl 3-methylbutanoate, resulting in enhanced peak (arrow).

95 (12), 93 (30), 81 (32), 79 (15), 69 (100), 67 (15), 55 (12), 53 (8), 43 (4), and 41 (38). These values agree with those reported for β-springene (Fig. 3), as originally characterized from the dorsal gland secretions of the springbok antelope (*Antidorcas marsupialis*, Mammalia) (Burger *et al.*, 1978) and subsequently observed in the paracloacal gland secretions of the smooth-fronted caiman (*Paleosuchus trigonatus*) (Avery *et al.*, 1993).

A COSY analysis of the diterpene hydrocarbon displayed absorptions consistent with β-springene; the ^{13}C NMR spectrum exhibits values within 0.1

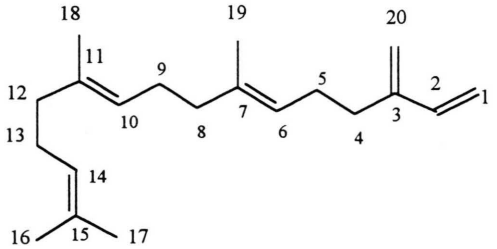

Fig. 3. The structural formula of β-springene, with carbons numbered for reference to Table II.

ppm of those reported for this isomer (Table II). Moreover, the catalytic hydrogenation of the isolated compound with Pd/Al_2O_3 yielded the reduction product expected for β-springene, which is 2,6,10,14-tetramethylhexadecane (phytane), with a molecular weight of 282 (M^+).

We observed trace amounts of two alcohols, dodecanol and tetradecanol, only in immature alligators. Both compounds were indicated by mass spectra displaying the following *m/z* values: 168 $(M-18)^+$, 140, 125, 111, 97, 83, 69, 55, and 41. The mass spectrum of tetradecanol also displays *m/z* values at 196 $(M-18)^+$ and 168 $(M-18-28)^+$. Weldon *et al.* (1988) did not observe alcohols in *A. mississippiensis*, but these compounds are documented in other alligatorids, including *A. sinensis* (Dunn *et al.*, 1993). The relative proportions of free alcohols observed in the secretions of adult *A. sinensis* were similar to those of the alcoholic functions of the acetates present (Dunn *et al.*, 1993), thus these alcohols could be derived from these esters. We failed to observe free alcohols representing the full spectrum of compounds esterified to fatty acids in the secretions of *A. mississippiensis*. Nonetheless, the two alcohols we observe in immature alligators may be related to the numerous dodecyl and tetradecyl esters that occur as major or minor components in this age-class.

Table II. ^{13}C and ^1H NMR assignments for β-springene. Carbon numbers are assigned as depicted in Fig. 3.

^{13}C Assignment	(ppm) Immature alligators	(ppm) Burger *et al.* (1978)	^1H Assignment	(ppm)
C_3	146.39	146.21		
C_2	139.13	139.07	H_2	6.40
C_{11}	135.45	135.41		
C_7	134.96	134.39		
C_{15}	131.12	131.21		
C_{14}	124.56	124.48	H_{14}	5.25
C_{10}	124.37	124.29	H_{10}	5.25
C_6	124.18	124.09	H_6	5.26
C_1	115.36	115.63	H_1	5.00
C_{20}	112.90	113.00	H_{20}	5.10
C_{12}	39.76	39.75	H_{12}	2.10
C_8	39.76	39.75	H_8	2.10
C_4	31.72	31.52	H_4	2.10
C_{13}	26.91	26.82	H_{13}	2.20
C_9	26.84	26.69	H_9	2.20
C_5	26.75	26.69	H_5	2.20
C_{16}	25.55	25.66	H_{16}	1.70
C_{17}	17.61	17.67	H_{17}	1.60
C_{18}	15.99	16.03	H_{18}	1.60
C_{19}	15.99	16.03	H_{19}	1.60

Weldon *et al.* (1988) reported only C_{16} and C_{18} FFA in samples of both immature and adult *A. mississippiensis*. We detected as methyl esters, among both immature and adult alligators, twelve FFA ranging in carbon-chain length from 7 to 18. Our ability to detect more FFA (primarily as trace components) than previously reported may be attributed to our analysis of these compounds as methyl ester derivatives. The mass spectra of all esters of saturated aliphatic acids show a diagnostic peak at *m/z* 74 $(CH_3OCOHCH_2)^+$ due to McLafferty rearrangement. In addition, all FFA display their molecular ions (M^+) and the ions at M-15 and M-31. We observed tetradecanoic and hexadecanoic acids in all samples of immature and adult alligators; the latter compound is a major component in immature alligators. Dodecanoic, octadecenoic, and octadecanoic acids were detected only in immature alligators. Octadecadienoic acid and 3,7,11-trimethyldodecanoic acid were observed only in adult alligators; the latter compound was characterized by the mass spectrum of its methyl ester derivative which showed a M^+ at 256, a rearrangement ion at *m/z* 74, and a fragment ion at *m/z* 69 (Fig. 4). One mixed-sex sample of adults contained heptanoic, undecanoic, and undecenoic acids. Odd-carbon FFA also have been reported in *A. sinensis* (Dunn *et al.*, 1993) and in caimans (*Paleosuchus* spp.) (Shafagati *et al.*, 1989).

Our comparison of the paracloacal gland lipids of the different age-classes of *A. mississippiensis* entailed analyses of samples from captive-raised (immature) and free-ranging (adult) individuals. We acknowledge that the disparate rearing conditions of secretion donors, especially their different diets, may have introduced uncontrolled variables confounding our results. Alligators of the two size-classes in our study also consume different prey

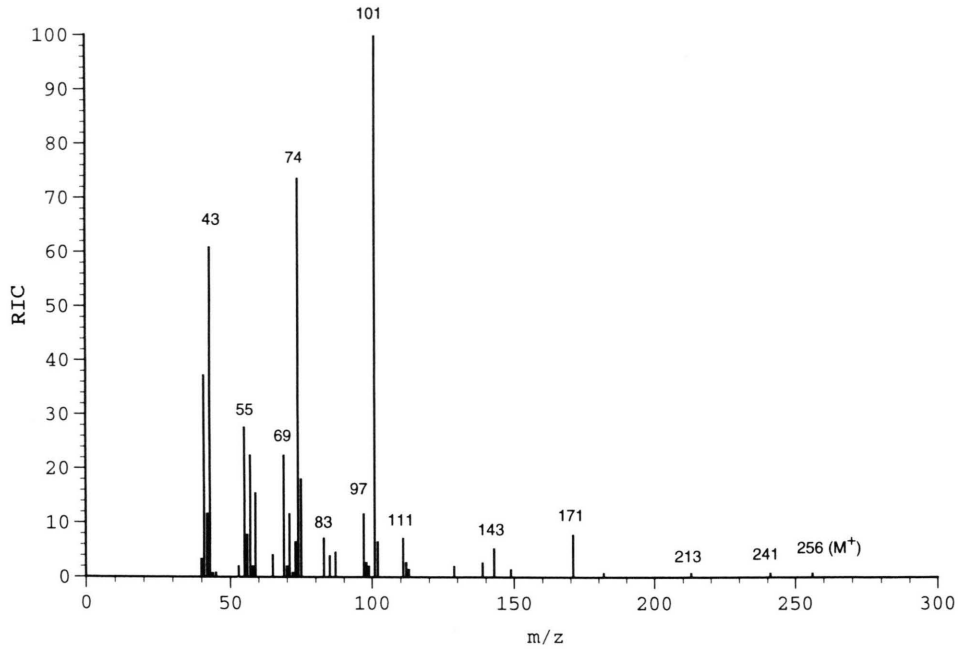

Fig. 4. Mass spectrum of methyl 3,7,11-trimethyldodecanoate, the methyl ester derivative of 3,7,11-trimethyldodecanoic acid.

in nature; immature alligators subsist on aquatic invertebrates, while adults consume a variety of vertebrates (Elsey *et al.,* 1992; Hayes, 1992). Nonetheless, the age-class differences we observe in the secretion composition of alligators from Louisiana are consistent with those reported by Weldon *et al.* (1988) and Shafagati (1989) for free-ranging alligators from Texas. Thus, we believe that these differences reflect ontogenetic changes in the lipid composition of the paracloacal gland secretions.

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