Journal of Chemical Ecology, Vol. 28, No. 4, April 2002 (© 2002)

REPTILIAN CHEMISTRY: VOLATILE COMPOUNDS FROM PARACLOACAL GLANDS OF THE AMERICAN CROCODILE (*Crocodylus acutus*)

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(Received April 16, 2001; accepted December 5, 2001)

Abstract—The secretion of the paracloacal glands of the American crocodile (*Crocodylus acutus*) contains over 80 lipophilic compounds, including saturated and unsaturated long-chain alcohols along with their formic, acetic, and butyric acid esters, and several isoprenoids. Most of these compounds were identified on the basis of mass spectra, obtained by GC-MS. In addition, identification of the major components was supported by infrared spectra obtained by GC-FTIR. Major differences are indicated in the composition of the paracloacal gland secretion of *C. acutus* and that of another crocodylid, the African dwarf crocodile (*Osteolaemus tetraspis*).

Key Words—American crocodile, *Crocodylus acutus*, paracloacal glands, fatty esters, formates, mass spectrometry, GC-FTIR.

INTRODUCTION

While insect chemical ecology has made great strides in the last five decades, much less is known about many comparable aspects of vertebrate chemical ecology (Eisner and Meinwald, 1995). The crocodilians, a small but fascinating group of ancient vertebrates, have aroused the curiosity of chemical ecologists in the last decade, with primary attention being paid to the chemical composition of their skin

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gland secretions (Weldon and Wheeler, 2000). The study of crocodilian ecology and behavior, however, has lagged behind that of most other tetrapods. This circumstance can be attributed in part to the difficulties inherent in studying responses to chemicals by these large and, for adults at least, dangerous reptiles in field or seminatural settings. We have studied the chemistry of the secretion produced in the paracloacal glands of the American crocodile (*Crocodylus acutus*, Crocodylidae) in the hope that knowledge of this chemistry will ultimately contribute to an understanding of crocodilian chemical ecology. These glands in crocodilians are thought to produce pheromones involved in the nesting and/or mating activities (Weldon and Ferguson, 1993).

Previous analyses of lipids from the paracloacal glands of crocodilians have occasionally led to the characterization of unusual compounds, thus reflecting some novel biosynthetic capabilities of these reptiles (Weldon and Wheeler, 2000). For example, a unique 19-carbon aromatic ketone, named dianeackerone, and a family of related steroidal esters were found to be major glandular products of the African dwarf crocodile (*Osteolaemus tetraspis*, Crocodylidae) (Whyte et al., 1999; Yang et al., 1999, 2000). Two stereoisomers of dianeackerone occur in varying ratios in adults of this species, but this compound was not detected in immatures.

We report here the results of an analysis by gas chromatography–mass spectrometry (GC-MS) of the paracloacal gland secretion of another crocodylid, *C. acutus*. This species occurs in freshwater and brackish coastal habitats in Mexico, Central America, northern South America, Cuba, Jamaica, Hispaniola, and in the tip of southern Florida. Our study revealed that the glandular secretion of this species is composed largely of formate, acetate, and butyrate esters of C_{12}–C_{20} straight-chain alcohols, along with the alcohols themselves. Interestingly, formate esters have not previously been reported in reptiles. This is the first detailed report of the skin gland lipids of *Crocodylus*, the most speciose genus of crocodilians.

METHODS AND MATERIALS

Paracloacal gland secretions of *C. acutus* were obtained from one adult male [total length (TL) = 3.0 m], one adult female (TL = 2.5 m), six juvenile males (TL = 1–1.5 m), five juvenile females (TL = 0.7–1.1 m), and five unsexed hatchlings (TL = 0.5–0.6 m) maintained at Cypress Gardens (Plant City, Florida), Gatorama (Palmdale, Florida), and the St. Augustine Alligator Farm (St. Augustine, Florida). The adults we sampled, which had been housed together for 2.8 years, are within the size range of reproductively active, free-ranging *C. acutus* (Kushlan and Mazzotti, 1989); they had copulated, although neither egg-laying nor nesting behavior had been observed. Most secretion donors hatched from eggs laid by captive females from Florida; one juvenile female was reared from an egg laid by a female from Jamaica. Animals at Cypress Gardens were fed rats and chicken necks, those at Gatorama were fed a commercial feline food

(Nebraska Brand, Central Nebraska Packing Company, North Platte, Nebraska) containing primarily horse meat, and those at the St. Augustine Alligator Farm were fed nutria (*Myocastor coypu*).

The secretions were collected by manually pressing the region around the cloacal opening, causing the walls of the cloaca to flare outward, exposing the openings of the gland ducts. Additional pressure applied to the glandular region resulted in the discharge of yellow secretions from the glands. These exudates were collected in capillary tubes that were broken off into glass vials. A few milliliters of CH_2Cl_2 were added to the vials, which were stored on dry ice.

Determination of Position of Double Bonds. Dimethyl disulfide (DMDS) derivatization was carried out according to a reported procedure (Attygalle and Morgan, 1986).

Reference Compounds. Fatty acids and alcohols, cholesterol, cholesta-3,5-diene, citronellol, pentadecane, and squalene were purchased from Sigma-Aldrich. Cholesteryl and alkyl formates were synthesized by the uncatalyzed reaction of each alcohol with an excess of formic acid (Burger et al., 1996). Acetates were synthesized by treating each alcohol with an excess of acetic anhydride and pyridine (Zhdanov and Zhenodarova, 1975). The reaction of the alcohols with butyryl, caproyl, and capryl chloride in CH_2Cl_2 afforded the corresponding butyrates, caproates, and caprylates, respectively (Ihara et al., 1990; Madden and Prestwich, 1994). 2,3-Dihydrofarnesol was prepared following a literature procedure (Kaplay et al., 1980).

Analytical Procedures. Gas chromatography was performed on a Hewlett-Packard (HP) 5890 instrument equipped with a splitless injector, and either a flame-ionization detector, a HP 5965A IR detector, or a HP 5970 mass selective detector. The samples were injected in the splitless mode. Analyses were performed using a 25-m $\times$ 0.22-mm fused silica-column coated with SE-54. The oven was programmed from 40°C (2 min) at 7°C/min to 280°C (30 min). To investigate the possible presence of nonvolatile compounds, we undertook a direct analysis of unfractionated secretion samples by NMR spectroscopy, as well as by electrospray ionization–mass spectrometry. No additional compounds were found with these methodologies.

RESULTS AND DISCUSSION

An exploratory survey of the paracloacal gland secretions of 21 crocodilians by thin-layer chromatography showed the presence of bands consistent with aliphatic esters in most taxa, including *Crocodylus* spp. (Weldon and Tanner, 1991). Analyses by GC-MS have revealed the structures of these esters in alligators (*Alligator* spp.) and caimans (*Caiman* spp. and *Paleosuchus* spp.) (Shafagati et al., 1989; Dunn et al., 1993; Ibrahim et al., 1998). The present study confirms the presence of many aliphatic acetates in *C. acutus*, and demonstrates the occurrence of formates (characterized by *m/z* 47 ions) and butyrates (characterized by *m/z* 89 ions) as well. Moreover, the ester derived from 1-hexadecyl alcohol is the main component of the caproic acid esters; 1-hexadecyl caproate (*m/z* 117 and 224 ions) is also abundant in the secretions of the Chinese alligator (*A. sinensis)* (Dunn et al., 1993). Our analytical results are presented in Tables 1 and 2 and Figures 1 and 2.

We observed significant quantities of formate esters derived from C_{13}–C_{18} alcohols; the major formates were those of C_{16}, C_{17}, and C_{18} monounsaturated alcohols. The presence of formates is interesting since they have not been reported previously from crocodilians or from any other reptiles. While formates are known from ants (Lopez et al., 1993), beetles (Attygalle et al., 1992), and caterpillars (Attygalle et al., 1993), to the best of our knowledge they have not been reported among vertebrates except in the preorbital gland secretions of three species of African antelopes (Burger et al., 1981, 1996, 1999; Mo et al., 1995). The alcoholic moieties of these esters range, among the three antelope species, from 9 to 28 carbons. As Burger et al. (1981) noted, the mass spectra of formates are very similar to those of the corresponding aliphatic alcohols, but are distinguished from them by the presence of a low-intensity peak at *m/z* 47, corresponding to an $HCO_2H_2^+$ (protonated formic acid) fragment.

We observed free alcohols in the *C. acutus* ranging from 10 to 18 carbons, including the terpene citronellol, which was found only in the secretion of the hatchlings. A similar array of alcohols has been reported in some alligatorids, e.g.,

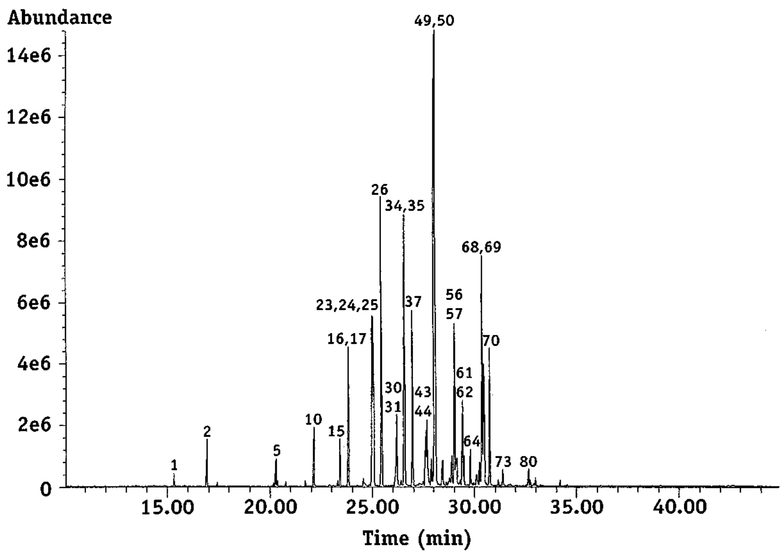

FIG. 1. A reconstructed gas chromatogram obtained from the paracloacal gland secretion of hatchling *Crocodylus acutus*. Peak numbers refer to components listed in Table 1.

TABLE 1. COMPONENTS FOUND IN PARACLOACAL GLAND SECRETION OF *Crocodylus acutus*[a]

No.	Compound	Molecular formula	NMM	Characteristic ions observed (*m/z*)	R_t (min)
1	Citronellyl formate	$C_{10}H_{19}OCHO$	184	138, 123, 95, 81, 69, 41	15.33
2	Citronellyl acetate	$C_{10}H_{19}O_2C_2H_3$	198	138, 123, 95, 81, 69, 43, 41	16.92
3	Pentadecane	$C_{15}H_{32}$	212	212, 71, 57, 43	20.11
4	Unidentified terpenoid			181, 135, 109, 69	20.17
5	Citronellyl butyrate	$C_{10}H_{19}O_2C_4H_7$	226	138, 123, 95, 81, 69, 43, 41	20.28
6	Dodecyl formate	$C_{12}H_{25}OCHO$	228	168	20.30
7	Tridecyl alcohol	$C_{13}H_{27}OH$	200	182	21.15
8	Dodecyl acetate	$C_{12}H_{25}OCHO$	228	168	21.71
9	Tridecyl formate	$C_{13}H_{27}OCHO$	228	182	22.12
10	Unidentified terpenoid			195, 123, 69, 41	22.19
11	Tetradecyl alcohol	$C_{14}H_{29}OH$	214	196	22.87
12	Unidentified terpenoid			209, 149, 123, 81, 69, 43, 41	23.30
13	Tetradecenyl formate[1]	$C_{14}H_{27}OCHO$	240	194	23.41
14	Tridecyl acetate	$C_{13}H_{27}O_2C_2H_3$	242	182	23.41
15	Unidentified terpenoid			209, 123, 69, 41	23.81
16	Tetradecyl formate	$C_{14}H_{29}OCHO$	242	196	23.81
17	Myristic acid	$C_{13}H_{27}CO_2H$	228	185	24.21
18	Unidentified terpenoid			195, 143, 171	24.21
19	Pentadecyl alcohol	$C_{15}H_{31}OH$	228	210, 182	24.52
20	Tetradecenyl acetate[1]	$C_{14}H_{27}O_2C_2H_3$	256	194	24.62
21	Unidentified		252	252, 135, 121, 79, 67,41	24.88
22	Dihydrofarnesyl acetate	$C_{15}H_{27}O_2C_2H_3$	266	223, 163109, 95, 81, 69, 43, 41	24.93
23	Tetradecyl acetate	$C_{14}H_{29}O_2C_2H_3$	256	196	25.01
24	Pentadecenyl formate[b]	$C_{15}H_{29}OCHO$	254	208	25.01
25	Pentadecyl formate	$C_{15}H_{31}OCHO$	256	210	25.42
26	Hexadecenyl alcohol[b]	$C_{16}H_{31}OH$	240	222	25.71
27	Pentadecanoic acid	$C_{14}H_{29}CO_2H$	242	199	25.71
28	Pentadecadienyl acetate[b]	$C_{15}H_{27}O_2C_2H_3$	266	206	25.97
29	Hexadecyl alcohol	$C_{16}H_{33}OH$	242	224	26.11
30	Pentadecenyl acetate[b]	$C_{15}H_{29}O_2C_2H_3$	268	208	26.18
31	Tridecyl butyrate	$C_{13}H_{27}O_2C_4H_7$	270	182	26.23
32	Unidentified		266	266, 135, 121, 81, 67, 41	26.39
33	7-Hexadecenyl formate[b]	$C_{16}H_{31}OCHO$	268	222	26.55
34	Pentadecyl acetate	$C_{15}H_{31}O_2C_2H_3$	270	210	26.55
35	Methyl palmitate	$C_{15}H_{31}CO_2CH_3$	270	227, 270	26.80
36	Hexadecyl formate	$C_{16}H_{33}OCHO$	270	224	26.94
37	Heptadecadienyl alcohol[b]	$C_{17}H_{31}OH$	252	234	27.15
38	Palmitic acid	$C_{15}H_{31}CO_2H$	274	256	27.22
39	Heptadecenyl alcohol[b]	$C_{17}H_{33}OH$	254	236	27.38
40	Heptadecyl alcohol	$C_{17}H_{35}OH$	256	238	27.48
41	Hexadecadienyl acetate[b]	$C_{16}H_{29}O_2C_2H_3$	280	220	27.48
42	Unidentified terpenoid			251, 191, 163, 81, 69	27.61
43	Hexadecenyl acetate[c]	$C_{16}H_{31}O_2C_2H_3$	282	222	27.61
44	Tetradecenyl butyrate[b]	$C_{14}H_{27}O_2C_4H_7$	282	194	27.64

TABLE 1. CONTINUED

No.	Compound	Molecular formula	NMM	Characteristic ions observed (m/z)	R_t (min)
45	Tetradecyl butyrate	$C_{14}H_{29}O_2C_4H_7$	284	196	27.69
46	Heptadecadienyl formate[b]	$C_{17}H_{31}OCHO$	282	234	27.78
47	Unidentified		280	280, 135, 121, 81, 67, 41	27.87
48	Hexadecyl acetate	$C_{16}H_{33}O_2C_2H_3$	284	224	28.02
49	Heptadecenyl formate[b]	$C_{17}H_{33}OCHO$	282	236	28.02
50	Heptadecyl formate	$C_{17}H_{35}OCHO$	284	238	28.42
51	Octadecenyl alcohol[d]	$C_{18}H_{35}OH$	268	250	28.70
					28.77
52	Pentadecenyl butyrate[b]	$C_{15}H_{29}O_2C_4H_7$	296	208	28.81
53	Unidentified terpenoid			169, 109, 69, 41	28.87
54	Heptadecadienyl acetate[b]	$C_{17}H_{31}O_2C_2H_3$	294	234	28.87
55	Heptadecenyl acetate[e]	$C_{17}H_{33}O_2C_2H_3$	296	236	29.02
					29.10
56	Octadecyl alcohol	$C_{18}H_{37}OH$	270	252	29.10
57	Pentadecyl butyrate	$C_{15}H_{31}O_2C_4H_7$	298	210	29.10
58	Unidentified		294	294, 135, 121, 81, 67, 41	29.28
59	Heptadecyl acetate	$C_{17}H_{35}O_2C_2H_3$	298	238	29.40
60	Octadecenyl formate[f]	$C_{18}H_{35}OCHO$	296	250	29.40
					29.48
61	Oleic acid	$C_{17}H_{33}CO_2H$	282	264	29.69
62	Octadecyl formate	$C_{18}H_{37}OCHO$	298	252	29.79
63	Stearic acid	$C_{17}H_{35}CO_2H$	284	266	30.07
64	Hexadecenyl butyrate[b]	$C_{16}H_{31}O_2C_4H_7$	310	222	30.07
65	Octadecadienyl acetate[b]	$C_{18}H_{33}O_2C_2H_3$	308	248	30.09
					30.24
66	Octadecenyl acetate[g]	$C_{18}H_{35}O_2C_2H_3$	310	250	30.36
					30.42
67	Hexadecyl butyrate	$C_{16}H_{33}O_2C_4H_7$	312	224	30.42
68	Octadecyl acetate	$C_{18}H_{37}O_2C_2H_3$	312	252	30.72
69	Unidentified		308	308, 135, 121, 81, 67, 41	31.17
70	Heptadecadienyl butyrate[1]	$C_{17}H_{33}O_2C_4H_7$	322	234	31.35
71	Heptadecenyl butyrate[b]	$C_{17}H_{33}O_2C_4H_7$	324	236	31.37
72	Pentadecyl caproate	$C_{15}H_{31}O_2C_6H_{11}$	328	177, 210	31.65
73	Nonadecenyl acetate[b]	$C_{19}H_{37}O_2C_2H_3$	324	264	31.66
74	Heptadecyl butyrate	$C_{17}H_{35}O_2C_4H_7$	326	238	31.73
75	Eicosenyl formate[b]	$C_{20}H_{39}OCHO$	324	278	32.00
76	Nonadecyl acetate	$C_{19}H_{39}O_2C_2H_3$	326	266	32.00
77	Octadecadienyl butyrate[b]	$C_{18}H_{33}O_2C_4H_7$	336	248	32.37
78	Octadecenyl butyrate[h]	$C_{18}H_{35}O_2C_4H_7$	338	250	32.64
					32.72
79	Eicosenyl acetate[b]	$C_{20}H_{39}O_2C_2H_3$	338	278	32.88
80	Hexadecyl caproate	$C_{16}H_{33}O_2C_6H_{11}$	340	177, 224	32.88
81	Octadecyl butyrate	$C_{18}H_{37}O_2C_4H_7$	340	252	32.98
82	Eicosyl acetate	$C_{20}H_{41}O_2C_2H_3$	340	280	33.23
83	Unidentified terpenoid			376, 333, 109, 69	34.18

TABLE 1. CONTINUED

No.	Compound	Molecular formula	NMM	Characteristic ions observed (m/z)	R_t (min)
84	Hexadecyl caprylate	$C_{16}H_{33}O_2C_8H_{15}$	368	368, 224, 145	35.13
85	Octyl oleate	$C_8H_{17}O_2C_{18}H_{33}$	394	264	37.52
86	Squalene	$C_{30}H_{50}$	410	41, 69, 81	38.09
87	Cholesta-3,5-diene	$C_{27}H_{44}$	368	368, 353, 147	39.06
88	Dodecyl oleate	$C_{12}H_{25}O_2C_{18}H_{33}$	450	264	40.70
89	Cholesterol	$C_{27}H_{46}O$	386	386	42.54
90	Cholesteryl formate	$C_{27}H_{45}OCHO$	414	368	43.95

[a] Compounds are numbered in order of increasing GC retention time. NMM = nominal molecular mass. Trace components are not listed.
[b] Double bond position undetermined.
[c] Mixture of (Z)-hexadec-7-en-1-yl acetate (major) and (Z)-hexadec-9-en-1-yl acetate (minor).
[d] Mixture of (Z)-octadec-9-en-1-ol (major) and (Z)-octadec-11-en-1-ol (minor).
[e] Mixture of (Z)-heptadec-8-en-1-yl acetate (major) and (Z)-heptadec-9-en-1-yl acetate (minor).
[f] Mixture of (Z)-octadec-9-en-1-yl formate (major) and (Z)-octadec-11-en-1-yl formate (minor).
[g] Mixture of (Z)-octadec-9-en-1-yl acetate (major) and (Z)-octadec-11-en-1-yl acetate (minor).
[h] Mixture of (Z)-octadec-9-en-1-yl butyrate (major) and (Z)-octadec-11-en-1-yl butyrate (minor).

the Chinese alligator (*A. sinensis*) (Dunn et al., 1993) and caimans (*Paleosuchus* spp.) (Shafagati et al., 1989), although citronellol has been described only from several caimans. 2,3-Dihydrofarnesyl acetate, a known paracloacal gland component of the brown caiman (*Caiman crocodilus fuscus*) (Wheeler et al., 1999), accounted for up to 4% of the secretion of immature crocodiles, but was not detected in the adult samples.

Interestingly, we did not find free formic, acetic, or butyric acids in any of the secretions, based on the derivatization of the total sample with pentafluorobenzyl bromide/triethylamine (Attygalle, 1998). Five C_{14}–C_{18} fatty acids, which are routinely encountered from vertebrate integumentary organs, were observed in immature *C. acutus*, and a significant amount of oleic acid (~5%) was found in the adult male; we failed to detect free fatty acids in hatchling *C. acutus*. Thirteen fatty acids ranging from C_{14} to C_{20} were reported from the skin glands of *Crocodylus* spp. by Navajas Polo et al. (1988a,b). It should be noted that these investigators combined, for their studies, the paracloacal and gular (throat) glands of both *C. acutus* and the Cuban crocodile (*C. rhombifer*) thus our results are not directly comparable with theirs. We were also unable to confirm the presence of an intriguing lactone related to 10-hydroxystearic acid described in an earlier report (Navajas Polo, 1982) from the macerated and steam-distilled skin glands of the aforementioned mixed species.

Squalene was the major hydrocarbon we detected in *C. acutus*. This compound was especially abundant in the adult female; it was either present in reduced

TABLE 2. RELATIVE AMOUNTS OF COMPOUNDS IDENTIFIED FROM PARACLOACAL GLANDS OF HATCHLING, IMMATURE, AND ADULT *Crocodylus acutus*[a]

		Immatures		Adults	
Compound	Hatchlings (5)	Female (5)	Male (6)	Female (1)	Male (1)
Alkyl and alkenyl esters					
Dodecyl formate	<0.2	<0.2	<0.2	ND	ND
Tridecyl formate	1.30	<0.2	0.32	ND	ND
Tetradecenyl formate	<0.2	<0.2	<0.2	ND	<0.2
Tetradecyl formate	2.07	0.24	0.73	ND	0.63
Pentadecenyl formate	1.10	<0.2	0.31	ND	0.30
Pentadecyl formate	8.16	1.09	2.91	ND	2.50
7-Hexadecenyl formate	1.30	0.57	1.83	0.51	2.07
Hexadecyl formate	4.44	1.53	4.92	0.96	6.89
Heptadecadienyl formate	ND	ND	<0.2	ND	ND
Heptadecenyl formate	4.12	2.53	0.77	<0.2	9.26
Heptadecyl formate	0.59	<0.2	0.91	ND	0.89
Octadecenyl formate	2.31	3.73	11.28	1.67	20.06
Octadecyl formate	0.99	0.61	2.62	1.39	4.80
Eicosenyl formate	ND	<0.2	<0.2	ND	<0.2
Dodecyl acetate	<0.2	<0.2	<0.2	ND	ND
Tridecyl acetate	1.02	0.45	0.31	ND	ND
Tetradecenyl acetate	<0.2	<0.2	<0.2	ND	ND
Tetradecyl acetate	3.82	1.65	1.14	<0.2	<0.2
Pentadecadienyl acetate	ND	<0.2	<0.2	ND	ND
Pentadecenyl acetate	1.90	0.64	0.71	ND	ND
Pentadecyl acetate	7.25	3.48	3.28	ND	0.86
Hexadecadienyl acetate	<0.2	<0.2	<0.2	ND	ND
Hexadecenyl acetate	2.06	1.21	1.72	0.97	0.61
Hexadecyl acetate	19.97	10.85	9.47	4.04	3.59
Heptadecadienyl acetate	1.02	2.02	1.79	ND	ND
Heptadecenyl acetate	5.36	6.13	8.90	2.51	4.31
Heptadecyl acetate	0.73	0.79	0.84	1.67	0.34
Octadecadienyl acetate	1.15	4.10	2.19	ND	6.09
Octadecenyl acetate	9.10	14.40	13.84	7.03	9.90
Octadecyl acetate	3.30	3.73	4.29	4.58	2.28
Nonadecenyl acetate	ND	<0.2	<0.2	ND	ND
Nonadecyl acetate	ND	<0.2	<0.2	ND	ND
Eicosenyl acetate	<0.2	<0.2	<0.2	ND	ND
Eicosyl acetate	<0.2	<0.2	<0.2	ND	ND
Tridecyl butyrate	<0.2	<0.2	0.23	ND	ND
Tetradecenyl butyrate	ND	ND	<0.2	ND	ND
Tetradecyl butyrate	0.32	0.36	0.54	<0.2	<0.2
Pentadecenyl butyrate	<0.2	ND	<0.2	ND	ND
Pentadecyl butyrate	0.60	0.93	0.74	ND	0.45
Hexadecenyl butyrate	<0.2	<0.2	0.28	ND	0.76
Hexadecyl butyrate	1.98	1.11	2.09	2.25	0.76
Heptadecadienyl butyrate	<0.2	<0.2	<0.2	ND	ND

TABLE 2. CONTINUED

Compound	Hatchlings (5)	Immatures		Adults	
		Female (5)	Male (6)	Female (1)	Male (1)
Heptadecenyl butyrate	0.422	0.33	0.69	ND	0.88
Heptadecyl butyrate	<0.2	<0.2	<0.2	ND	0.42
Octadecadienyl butyrate	ND	<0.2	<0.2	ND	ND
Octadecenyl butyrate	0.61	0.64	0.98	<0.2	3.30
Octadecyl butyrate	0.20	0.40	<0.2	<0.2	0.94
Pentadecyl caproate	<0.2	<0.2	ND	ND	ND
Hexadecyl caproate	<0.2	<0.2	<0.2	ND	ND
Hexadecyl caprylate	ND	<0.2	ND	ND	ND
Methyl palmitate	ND	<0.2	<0.2	ND	ND
Octyl oleate	ND	ND	<0.2	ND	ND
Dodecyl oleate	ND	<0.2	<0.2	ND	ND
Terpenoid Esters					
Citronellyl formate	0.26	ND	ND	ND	ND
Citronellyl acetate	1.05	ND	ND	ND	ND
Citronellyl butyrate	0.66	ND	<0.2	ND	ND
Dihydrofarnesyl acetate	4.75	0.63	0.41	<0.2	ND
Alcohols					
Tridecyl alcohol	ND	<0.2	ND	ND	0.22
Tetradecyl alcohol	ND	0.23	<0.2	ND	ND
Pentadecyl alcohol	0.23	1.16	0.68	ND	ND
Hexadecenyl alcohol	ND	<0.2	<0.2	ND	ND
Hexadecyl alcohol	0.58	2.30	1.24	ND	ND
Heptadecadienyl alcohol	ND	<0.2	0.26	0.26	ND
Heptadecenyl alcohol	<0.2	0.26	1.23	ND	ND
Heptadecyl alcohol	ND	<0.2	<0.2	ND	ND
Octadecenyl alcohol	0.46	1.00	2.30	ND	ND
Octadecyl alcohol	ND	0.37	<0.2	ND	ND
Acids					
Myristic acid	ND	<0.2	<0.2	ND	ND
Pentadecanoic acid	ND	<0.2	<0.2	ND	ND
Palmitic acid	ND	<0.2	0.35	ND	ND
Oleic acid	ND	0.22	0.88	ND	4.89
Stearic acid	ND	<0.2	<0.2	ND	ND
Hydrocarbons					
Pentadecane	ND	<0.2	ND	ND	ND
Squalene	ND	3.44	0.74	52.00	0.33
Steroids					
Cholesta-3,5-diene	ND	<0.2	<0.2	ND	ND
Cholesterol	ND	0.27	<0.2	ND	<0.2
Cholesteryl formate	ND	<0.2	<0.2	ND	ND
Unidentified compounds					
Terpenoid No. 4	<0.2	0.45	<0.2	<0.2	ND
Terpenoid No. 10	<0.2	<0.2	<0.2	ND	ND
Terpenoid No. 12	<0.2	<0.2	<0.2	ND	ND

TABLE 2. CONTINUED

Compound	Hatchlings (5)	Immatures Female (5)	Immatures Male (6)	Adults Female (1)	Adults Male (1)
Terpenoid No. 15	1.37	0.27	0.52	ND	ND
Terpenoid No. 18	ND	ND	<0.2	ND	ND
Unidentified No. 21	ND	<0.2	<0.2	ND	ND
Unidentified No. 32	0.25	<0.2	0.27	ND	ND
Unidentified No. 42	1.50	<0.2	<0.2	ND	ND
Unidentified No. 47	0.78	0.71	1.61	ND	2.46
Unidentified No. 53	<0.2	<0.2	ND	ND	ND
Unidentified No. 58	<0.2	0.54	0.77	ND	3.00
Unidentified No. 69	<0.2	<0.2	<0.2	ND	ND
Terpenoid No. 83	<0.2	ND	<0.2	ND	ND

[a] Numbers in parentheses indicate the number of individuals sampled. ND = non detected.

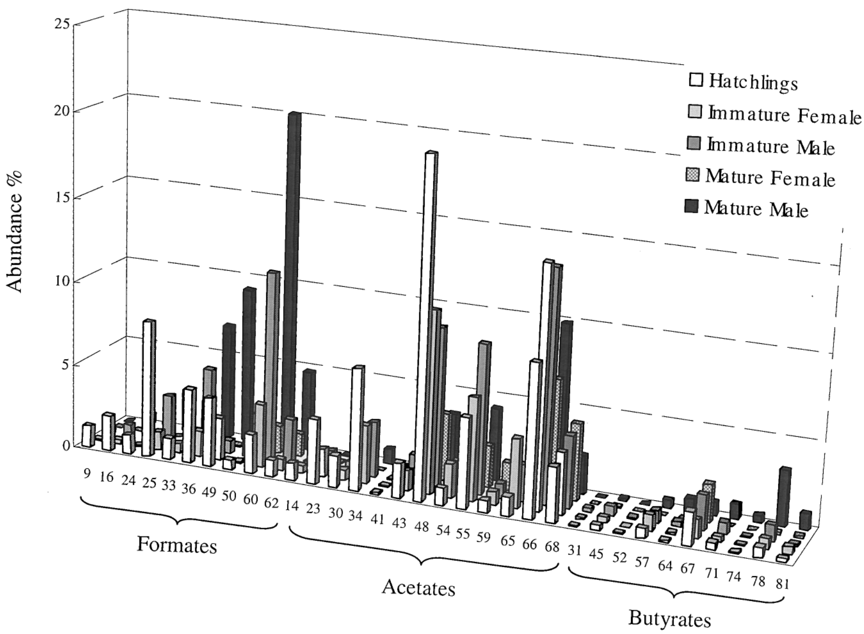

FIG. 2. Distribution of the alkyl and alkenyl esters (formates, 9–62; acetates, 14–68; and butyrates, 31–81) found in average abundance >0.2% in the paracloacal gland secretion of *Crocodylus acutus*, expressed as a percentage of the total secretion. Percentages are listed in Table 2; numbers refer to components listed in Table 1. Crocodile hatchlings were not sexed.

quantities or was entirely absent in the male or immature females. The abundance of squalene in our adult female is consistent with the appearance of an intense band corresponding to hydrocarbons in the thin-layer chromatogram of a female *C. acutus* secretion sample examined in the earlier survey of paracloacal gland lipids by Weldon and Tanner (1991).

All our compound identifications are based on the mass spectra obtained by GC-MS and comparison with synthetic standards. In addition, the identification of the major components was supported by infrared spectra obtained by gas chromatography–Fourier transform IR spectroscopy. Gas-phase FTIR spectra proved particularly helpful in recognizing formate, acetate, and butyrate peaks. Thus, the carbonyl group stretching vibrations appeared characteristically at 1740–1748 cm^{-1} in the gas-phase FTIR spectra of C_{13}–C_{18} formates, whereas that of acetates and butyrates appeared at 1761–1762 and 1753–1754 cm^{-1}, respectively. FTIR spectra were also useful for the assignment of the *Z* configuration to the double bonds in the unsaturated esters and alcohols. Double-bond positions in 9- and 11-octadecenyl acetate, 8- and 10-heptadecenyl acetate, 7- and 9-hexadecenyl acetate, 9-octadecenyl formate, 8-heptadecenyl formate, and 7-hexadecenyl formate were determined by the DMDS derivatization method (Buser et al., 1983; Francis and Veland, 1981).

We did not observe in *C. acutus* either dianeackerone or the related steroidal esters characteristic of the confamilial *Osteolaemus tetraspis* (Whyte et al., 1999; Yang et al., 1999); conversely, neither the aliphatic esters, alcohols, nor squalene that we have observed in *C. acutus* have been found in *O. tetraspis*. These results, foreshadowed by earlier TLC studies (Weldon and Tanner, 1991; Weldon and Wheeler, 2000), demonstrate substantive chemical differences in the paracloacal gland products of *Crocodylus* and *Osteolaemus*. While further analyses of the glandular products of *Crocodylus* will be needed to assess the range of chemical variation within the Crocodylidae, we can anticipate that the deeper task of deciphering the biological significance of these chemically complex secretions will remain a challenge for some time to come.

Acknowledgments—We are grateful to K. Bartel, B. Schwedick (Cypress Gardens, Plant City, Florida), A. Register, P. Register (Gatorama, Palmdale, Florida), J. Brueggen, J. Darlington, J. Heil, S. Huber, D. Kledzik, W. Puckett, and T. Rexroad (St. Augustine Alligator Farm, Florida) for allowing access to animals in their collections. The partial support of this research by a grant from the Research Corporation (DS0260), as well as a postdoctoral fellowship from the Pew Latin American Fellows Program in the Biomedical Sciences (P0236SC) to S.G.R., is acknowledged with pleasure.

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