Natural products from the integument of nonavian reptiles

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This review describes the epidermal and glandular chemistry of nonavian reptiles in relation to proposed functions, and includes more than 170 references. The results are presented according to the different reptile taxa.

1 Introduction

The appearance of the amniote egg during the Carboniferous period 350 million years ago (mya) marked the emergence of the first fully terrestrial vertebrates and led to the evolution of reptiles. Reptilian diversity expanded dramatically during the Mesozoic era (245–65 mya), the so-called “Age of Reptiles”, with radiations of flying, marine, semiaquatic, and various terrestrial forms, including dinosaurs. Five reptile orders from the Mesozoic have survived: Squamata (amphisbaenians, lizards, and snakes), Rhynchocephalia (tuatara), Testudines (turtles), Crocodylia (alligators and caimans, crocodiles, and gavials), and Aves (birds) (Fig. 1). Together, these taxa comprise the most speciose assemblage of extant tetrapods, with >16000 species occupying diverse habitats worldwide.

The evolutionary success of reptiles is due, in part, to their possession of an integument that restricts the loss of water to the environment. Cutaneous water conservation is achieved by a multilayered stratum corneum, the outermost region of dead epidermis, that is imbued with lipids.1–4 These lipids establish the transepidermal permeability barrier, impeding desiccation and the percutaneous inward passage of substances from the environment. Chemicals from the integument – the epidermis and skin glands – also protect reptiles against pathogenic microorganisms,5 ectoparasites, including disease vectors,6 and predators,7 in addition to attracting mates and eliciting other pheromonal responses.8

Here, we describe chemicals from the integument of nonavian reptiles, their specific sources, and possible significance as skin products. Although we do not treat birds in detail, we refer broadly to what is known of the skin chemistry of tetrapods in examining primarily lipids and other low molecular weight compounds from squamates, tuatara, turtles, and crocodylians. The results of both preliminary analyses, as by thin-layer...
chromatography (TLC), and detailed structural identifications by modern analytical methods (GC, HPLC, MS, and NMR) are summarized. We also describe what is known of proteins from the integumental glands of reptiles in order to draw attention to these poorly understood skin products.

The following section of this paper describes compounds from the epidermis of squamates, the sole group of reptiles for which the chemistry of this outermost skin layer has been detailed. Subsequent sections describe chemicals from the skin glands of each extant reptile order where secretions have been investigated beyond basic histochemistry. We refer to compounds for which characterizations are reliably described, omitting those with invalid names or very unlikely natural occurrence. In the final section, we discuss the diversity and possible adaptive trends exhibited by natural products from the integument.

2 Epidermis: Squamata

TLC and other general analyses of lipids from intact or shed skins of lizards and/or snakes suggest the presence of hydrocarbons, free fatty acids (FFAs), alcohols, aldehydes, methyl ketones, di- and triacylglycerols, wax esters, sterols and their esters, phospholipids including sphingomyelin, and glycolipids. Taxonomic, sexual, seasonal, individual, and mutational variation in epidermal lipids have been described.

Squalene (1) has been observed in the skins of snakes and lizards. This compound occurs in male red-sided garter snakes (Thamnophis sirtalis parietalis) from Canada, but is present in reduced amounts or absent in females and female-mimicking males (“she-males”). Mason et al. observed that courtship behaviors experimentally elicited in male garter snakes by hexane skin extracts of females were inhibited by 1. These investigators posited that 1, along with other unidentified components, contributes to the chemosensory recognition of male garter snakes by conspecifics.

Hydrocarbons reported from the skin of the Burmese python (Python molurus bivittatus) include a series of unbranched C_{14–C_{31}} alkanes and mono-, di-, trimethyl- and phenylalkanes as well as alkenes (all uncharacterized). Alkanes with up to 35 carbons occur in the eastern indigo snake (Drymarchon corais) and the leopard gecko (Eublepharis macularius). The indigo snake compounds, however, exhibited GC elution patterns deemed typical of petroleum hydrocarbon contaminants. Disagreement exists on whether hydrocarbons naturally occur on the skin surface of terrestrial vertebrates. Their presence on the epidermis may vary among species.

Cholesterol (6), which is ubiquitous in the tissues of tetrapods, is abundant on the epidermis of squamates. Cholesterol comprises 15% by weight of shed skin extracts. Ball observed proportionally more 6 in the skins of hatchling cornsnakes (Pantherophis guttata) than in those of

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mostly odd-numbered carbon chains ranging from C29 to C31 secondary alcohols. Some of these sterols, as well as stigmasterol (33), ergostanol (34), ergostanol (36), stigmastanol (42), β-sitosterol (43), stigmasterol (46), and fucosterol (48), are typical microbial or plant products. Their occurrence in snakes, which are strictly predatory, is noteworthy. Compound 2, which was reported from the skin of the cornsnake, is a component of bile.

FFAs and fatty acids bound in triglyceric acids, wax esters, sterol esters, and other compounds are widespread on the skin of tetrapods. The FFAs reported from squamates contain chains with up to twenty-eight carbons, the most abundant of which are common C16 and C18 compounds. Methyl-branched and hydroxylated FFAs also occur. Interspecific differences likely exist among snakes in FFAs of the epidermis. However, the different methods of extraction, storage, and analysis used in different studies, the small sample sizes typically involved, and the general failure to control for sex, age class, and other variables that could influence skin lipid composition preclude rigorous interspecific comparisons. The Japanese snakes, the habu (Protobothrops flavoviridis, Vipersidae) and two ratsnakes (Elaphe climacophora and E. quadravirgata, Colubridae), exhibit familial differences in the composition of epidermal FFAs.

Esterified saturated and monounsaturated C14 and C16 acids were observed among the triacylglycerols and mono- and diester waxes from the skin of the Burmese python. The monoester waxes of this snake contain saturated C16–C20 and C28 primary alcohols and C21, C23, C27, C29, and C31 secondary alcohols. C14–C24 acids occur among the triacylglycerols, sterol esters, and polar lipids of the black ratsnake (Pantherophis obsoleta). Common C16 and C18 acids are prominent esterified components among squamates, but the abundances of different acids may vary among compound classes. Studies of these compounds may shed light on their relative contribution (via hydrolysis) to the pool of FFAs on the skin surface.

Methyl ketones are reported from the epidermis of some colubrid snakes and the leopard gecko. On the basis of their investigation of the eastern indigo snake, Ahern and Downing postulated that methyl ketones arise from FFAs that have undergone β-oxidation followed by decarboxylation. Female red-sided garter snakes possess a series of saturated and Z-monounsaturated methyl ketones and mostly odd-numbered carbon chains ranging from C20 to C26. Bioassays revealed that males attend to these compounds to recognize and trail prospective mates. The unsaturated methyl ketones, which are more attractive to males, possess a double bond at the position. Unsaturation at this site denotes compounds potentially derived from (Z)-octadeceno (oleic) acid. Methyl ketones also occur in male and she-male garter snakes.

Subtle variation in the composition of methyl ketones on the skin surface of female garter snakes permits males to distinguish between large and small, less preferred mates. Large females primarily possess monounsaturated methyl ketones, whereas small females primarily possess saturated analogs. Males also rely on methyl ketone profiles to discriminate between females from their own versus foreign dens, preferring the former as mates. Females from different dens possess different proportions of monounsaturated methyl ketones, whereas the amounts of saturated analogs they possess are more uniform.

Nuclear magnetic resonance (13C) spectra of skin extracts of females of the eastern indigo snake, the common kingsnake (Lampropeltis getula), and the tropical ratsnake (Spilotes pullatus) exhibited signals denoting methyl ketones. The eastern indigo snake possesses saturated C21–C25 methyl ketones and C25–C35 analogs unsaturated at the position that probably are derived from (Z)-9-hexadecenoic (palmitoleic) acid. One isomeric pair each of C35, C36, and C37 ketodienes (66–71) showing (6Z, 8−9) or (8Z, 8−9) arrangements of double bonds were identified from females of the brown treesnake (Boiga irregularis), a rear-fanged constrictor accidentally introduced into Guam, where it has exterminated some native birds and threatens other wildlife. These ketodienes and the other methyl ketones present in this species (52–59, 61–65), if active as pheromones, might be used to control this invasive snake. Female leopard geckos possess saturated methyl ketones and as well as uncharacterized unsaturated analogs, but males do not.

Monounsaturated C23–C33 primary alcohols and C25–C35 secondary alcohols occur in the eastern indigo snake. The secondary alcohols exhibit carbon-chain lengths corresponding to those of the methyl ketones present, thus pointing to a close biosynthetic relationship between these compound classes. Ahern and Downing postulated that the methyl ketones in this snake undergo oxidation to form acetates that give rise to primary alcohols, and that secondary alcohols arise by the reduction of methyl ketones.

Roberts also observed alcohols in the black ratsnake, but failed to observe ketones in this or the other squamates she examined by TLC. Ball observed C12–C25 alcohols in the cornsnake, but methyl ketones were not detected. Higher concentrations of octadecanol were found in the skins of adult cornsnakes than in hatchlings, a contrast that Ball attributed to age-class differences in diet or lipid metabolism. Tetradecanal was also found in the cornsnake.

Glycolipids are essential to maintaining the transepidermal water barrier of amniotes. These compounds are believed to act as molecular rivets, stabilizing the intercellular lipid lamellae of the stratum corneum and obstructing the passage of water. In mammals, acylglucosylceramides are critical to the integrity of the transepidermal water barrier, whereas in reptiles, including birds, sterol glycosides serve this role.

Two classes of sterol glycosides were isolated from the skin of the bullsnake (Pituophis catenifer sayi), a sterol-β-glucoside and acylglucosylsterols with acyls acylated at C-6 of glucose. The acyl parts of the latter compounds consist of fatty acids with different chain lengths, primarily common C14 and C18 acids. These sterol glycosides are similar to those identified from the epidermis of the chicken (Gallus domesticus). Cholesterol is the sole sterol in the snake-derived glycosides, whereas the chicken-derived compounds contain either cholesterol or cholesterol; the latter is a primary sterol of the avian epidermis. The prevalence of cholesterol in the integument of birds, often overshadowing cholesterol as the chief skin sterol of tetrapods, has been related to waterproofing the plumage, although it is unclear if cholesterol is better suited for this function.
3 Integumentary glands: Squamata

3.1 Amphisbaenians

3.1.1 Precloacal gland. Amphisbaenians are elongate, burrowing reptiles whose limbs are absent or reduced. They inhabit loose or sandy soils in tropical and warm temperate regions around the world. Most amphisbaenians possess precloacal glands, narrow tubes embedded in the dermis that open through a semicircular series of pores anterior to the vent.\textsuperscript{9,10,43} Secretions from these glands, which are deposited by
abrasion as animals crawl through their tunnels, contain pheromones involved in sexual and/or individual recognition. An analysis of the precloacal gland secretions of Blanus cinereus from Spain revealed that cholesterol (6) and cholesteryl methyl ether (19) are the main lipid components. Other steroids found include 13, cholesta-4,6-dien-3-ol (14), cholesta-5,7-dien-3-ol (15), cholesta-5,7,9(11)-trien-3-ol (16), 3-methoxy cholestane (18), cholesta-5-en-3-one (23), and γ-sitosterol (44), as well as cholest-5-en-3-yl tetradecanoate (7) and cholesta-5,7-dien-3-yl acetate (20). FFAs in the secretions range in chain length from C₉ to C₁₈, the most abundant of which are dodecanoic and hexadecanoic acids. A C₁₈ methyl ester and C₁₆ hexanoates and octanoates also were observed. Squalene (1) occurs chiefly in the secretions of males. α-Tocopherol (72, vitamin E), which typically is produced by microorganisms and plants, and thus may be dietary in origin, was detected only in females. This compound is a radical scavenger and may protect other compounds in the secretions from oxidation. Sexual differences also were observed in the occurrence of some FFAs and steroids.

3.2 Lizards

3.2.1 Femoral, precloacal, and preanal glands. Lizards, overall, possess an array of glands, variously referred to as femoral, precloacal or preanal glands, that open onto the skin surface near the vent or on the thigh. These organs typically produce secretions that protrude through pores as solid plugs. They are most active in males during the mating season and generally are believed to produce pheromones for sexual signaling and/or territorial scent marking.

TLC analyses of the preanal gland secretions of the Indian house lizard (Hemidactylus flaviviridis, Gekkonidae) and Hardwick’s spiny-tailed lizard (Uromastix hardwickii, Agamidae), both obtained commercially, suggested the presence of FFAs, triacylglycerols, wax esters, sterols and their esters, and phospholipids in males of both species. Only the last three compound classes were observed in female spiny-tailed lizards; female house lizards lack preanal glands. Histochemical studies of males of both lizards revealed that the lipid content and various enzymatic activities in the glandular tissues and/or secretions peak during the mating season. An increase in enzyme activity associated with the citric acid cycle may reflect enhanced lipogenesis via acetyl CoA carboxylase.

The femoral gland secretions of males of the green iguana (Iguana iguana), a neotropical herbivorous iguanid, were found to contain FFAs and/or esterified fatty acids with chain lengths between C₁₄ and C₂₆; the steroids 3, epicerostanol (4), 6, lanosterol (29), 33, 43, stigmasterol (46); and TLC components consistent with triacylglycerols, methyl esters, sterol esters, and phospholipids. The steroids 33, 43, and 46 and their respective esters comprise about 10% of the total glandular lipids. These typical phytosterols may be derived from the diet.

Alberts et al. found that the femoral gland secretions of male green iguanas during the mating season contain an elevated lipid content and a greater abundance of unsaturated acids among the FFAs, triacylglycerols, and methyl esters. This seasonal variation may enhance the volatility, and thus the detectability, of scent deposits. The sterol content of the secretions and, to a lesser degree, that of the acids vary among individuals. A qualitative comparison of femoral gland lipids from juvenile and adult green iguanas failed to indicate differences between them, however, these age classes differed by only one year.

The femoral gland secretions of males of the Iberian rock lizard (Lucartea monticola cyreni), a montane lacertid from the Iberian Peninsula, contain 1, C₆–C₂₂ FFAs, C₁₈–C₂₆ primary alcohols, methyl decanoate, methyl eicosanoate, ethyl hexadecanoate, isopropyl dodecanoate and tetradecanoate, as well as the lactone 4-hexadecanolide (74). The steroids present include 6, cholesta-2,4-diene (12), 13, 14, 33, ergosta-5,8-diene-3-ol (37), ergosta-5,22-dien-3-ol (39), ergosterol (41), γ-sitosterol (44), stigmasta-5,24(28)-dien-3-ol (47), 24-propylidenecholesterol-5-en-3-ol (49), and several 4,4-dimethyl triterpenoids such as lanost-8-en-3-ol (28), 4,4-dimethylcholesterol-7-en-3-ol (30), 4,4-dimethylcholesta-5,7-dien-3-ol (31), as well as cholesta-5,7-dien-3-ol (15 dehydrocholesterol), a precursor of vitamin D₃ that is essential for calcium uptake and bone deposition. Alkanes also may be present. The concentrations of some femoral gland components in male rock lizards are positively correlated with features associated with social dominance and mate attractiveness. For example, large males contain high proportions of sterols, such as 6, 33, and 44, as well as some FFAs, such as nonanoic, decanoic, and octadecanoic acids. High quality males, identified experimentally by their superior T-cell-mediated immune response and other indicators, were found to contain high proportions of 15 and 41.
in their secretions. The concentration of 15 increased in males receiving it as a dietary supplement.  

Female rock lizards exhibited heightened tongue flicking to cotton swabs treated with the secretions of males containing high amounts of 15 and 41 and to swabs treated with solutions of these authentic compounds.  

Females also were attracted to areas scent-marked by these males, thus implicating 15 and 41 in mediating mate choice. Cholesterol (6) in the secretions of male rock lizards reportedly signals their fighting ability.  

The femoral gland secretions of male Psammodromus algirus, a Mediterranean lacertid inhabiting forests and pastures, were found to contain squalene (1), C16 and C18 alkanes, FFAs with chain lengths between C8 and C22, as well as methyl eicosatetraenoate, hexadecyl octadecenoate, octadecyl hexadecenoate, 1-octanol, a bishomologous series of C16–C22 primary alcohols, as well as saturated and unsaturated C7–C12 aldehydes, 72, and 74.  

The steroids present include 6, 13, 15, cholesta-5,22-dien-3-ol (17), cholesta-4-en-3-one (22), 4-methylcholest-7-en-3-ol (26), 27, lanost-8-3-en-3-ol (28), 29, 30, 31, 33, 34, γ-ergostenol (36), 37, 39, ergosta-7,22-dien-3-ol (40), 41, 44, stigmaster-7-en-3-ol (45), and 46. Campsterol (33) is the chief sterol in the glandular secretions of Psammodromus algirus, whereas 6 is the primary sterol in the secretions of many other lizards. Most of the compounds identified in this species occur in both juveniles and adults; however, the two wax esters listed above occur only in adults and 22 occurs only in juveniles. Martin and López postulated that conspecifics derive information on the age of males from the proportions of these compounds in their secretions. 

The femoral gland secretions of males of Schreiber’s green lizard (Lacerta schreiberi), which occurs in moist, woody habitats of the Iberian Peninsula, contain 1, C9–C29 FFAs, C12–C24 alcohols, 2-pentadecanoic and 2-hexadecanone, 72, methyl 4-hydroxyoctadecanoic, ethyl eicosatetraenoate, and γ-lactones of C16 and C18 hydroxy acids.  

The steroids present include 3, 6, 8, 13, 19, cholestan-3-one (21), cholesta-3,5-dien-7-one (25), 26, 27, 4,4-dimethylcholesta-8,14-dien-3-ol (32), 33, 34, 36, ergost-22-en-3-ol (38), 44, 45, 3,11-dihydroxy pregnan-20-one (50), and 20-methylnorgluc-9-en-3-one (51).  

The femoral gland secretions of male Iberian wall lizards (Podarcis hispanica, Lacertidae) and common wall lizards (Podarcis muralis), both collected in Spain, contain squalene (1), and tetramethylheaxadecapentaene, probably the diterpene β-springene (129), FFAs and alcohols with chain lengths between C8 and C29 and several wax-type esters comprised of them, ethyl and isopropyl esters, 72, nonanal, nonadecanol, and 74.  

The steroids present include 3, 6, cholest-5-en-3-yl acetate (8), 13, 14, 15, 16, 22, cholesta-4,6-dien-3-one (24), 31, 33, 34, 37, 41, 44, 46, and 49. Forty of seventy compounds are shared by both species. Twenty compounds are unique to Iberian wall lizards, whereas eight compounds are unique to common wall lizards.  

Female wall lizards in choice tests preferred the scent of males whose secretions contained a high content of 15 and a low content of 6.  

The proportions of 15 in the secretions of males correlated positively with their T-cell-mediated immune response, suggesting that this compound denotes high quality potential mates.  

The femoral gland secretions of males of the spiny-footed lizard (Acanthodactylus erythrurus), a lacertid that inhabits dry, sparsely vegetated habitats in Western Europe, contain 1, C9–C20 FFAs, C10–C29 alcohols, C13 and C16 ketones, hexadecyl hexadecenoate and octadecyl octadecenoate, a C18 hydroxylated methyl ester, ethyl eicosatetraenoate, isopropyl tetracanoate, 72, and the γ-lactones 4-dodecanolide (73), 74, and 4-octadecanolate (75).  

FFAs with chain lengths between C9 and C15 were observed chiefly in subadult males, whereas C9 and C16 were observed chiefly in adults. López and Martin suggested that the higher molecular weight acids in adult lizards enhance the persistence of their territorial scent marks in the dry environments they inhabit. The steroids present include 6, 13, 16, 31, 33, 37, 44, and 15 and its acetate.  

Environmental variables may influence the nature of chemicals used as pheromones by terrestrial vertebrates. Higher temperatures, of course, increase the rate at which compounds evaporate from scent marks, thus selecting for higher molecular weight semiochemicals. Humidity also may influence volatility.  

Martin and López investigated the possible influence of habitat humidity on the femoral gland chemistry of Iberian wall lizards. Lizards from one population (type 1) that typically occurs in the humid highlands of northwestern Iberia were compared with those from a population (type 2) that typically occurs in the arid Mediterranean region of central and southern Iberia. The lizards used in this study were from overlapping populations in central Spain. Type 1 males were found to possess twelve compounds not detected in type 2 males, including wax esters. Martin and López concluded that the different chemical profiles of the two lizard types are related to the different climatic conditions of the geographic areas they occupy, where less volatile and more stable femoral gland compounds occur in humid habitats. However, the subjects used by Martin and López originated from the same area. The adaptive significance of the contrasting lipid profiles of these lizards, therefore, is unclear, unless gene flow or other mitigating circumstances prevail.  

The precloacal gland secretions of twenty Chilean lizards of the genus Liolaemus (Tropiduridae) collectively contain C10–C29 alkanes, butanedioic and hexanedioic acids, as well as lactic acid. Fatty acids with chain lengths between C6 and C26, and some methyl esters derived from them, are also present. The steroids present include 3, 5, 6, 11, 41, and 43. Cholesterol and C14-C18 FFAs and/or esterified fatty acids occur in all species. One species examined in detail, Liolaemus bellii, was found to exhibit individual variation among the alkanes, carboxylic acids, and sterols in the secretions, prompting Escobar et al. to postulate that precloacal glands secrete self-recognition pheromones.  

A comparison of two genetically distinct populations of Liolaemus fabiani inhabiting the Atacama Salt Flat in Chile revealed minor populational differences in precloacal gland lipids. Cholesterol and hexanoic acid, the most volatile acid observed in this species, are more abundant in the population.
exposed to higher temperatures. Escobar et al. proposed that acts as an unreactive matrix, reducing the volatilization or degradation of secretion-borne semiochemicals, thus preventing their loss at high temperatures.

They also hypothesized that Liolaemus spp. occupying higher elevations and lower latitudes adapt to these environments by producing more precoloal gland secretions and/or less volatile secretion components. They observed that the number of precoloal gland pores present in Liolaemus spp. correlated positively with elevation and negatively with latitude. Escobar et al. concluded that lizards adapt to harsh environments by producing more secretions, although they did not measure secretion output in their comparative study. Furthermore, comparisons among forty-nine compounds from the twenty Chilean lizard species they analyzed failed to indicate correlations between secretion composition and environmental variables.

Recently, the femoral gland secretions of the sungazer (Cordylus giganteus, Cordylidae), a large lizard endemic to South African grasslands, were found to contain 1, pentacosane, C14–C24 FFAs, 1-dodecanol and 1-hexadecanol, 2-heptadecenal, as example, proteins comprise the urodeum, via urodeal glands that empty into folds of the urogenital chamber, Scincidae, and other families possess tubular organs called exudates when conspecifics investigate them by tongue flicking. 6 They demonstrated that the secretions of female brown treesnakes (Crotalus spp.) and some other crotalines, defensively spray scent gland fluids. Gel electrophoresis of the secretions of the spiny-tailed gecko (Diplodactylus ciliarius), Rankin’s spiny-tailed gecko (Diplodactylus rankini), and the silver spiny-tailed gecko (Diplodactylus strophurus) revealed in each species three major proteins of masses ca. 30, 45, and 80 kDa. Only the 80 kDa component of the silver spiny-tailed gecko reacted positively for glycoprotein.

3.3 Snakes

3.3.1 Scent gland. All snakes possess in the base of their tail a pair of elongate sacs known as scent glands that open through two ducts exiting at the posterolateral margin of the vent. Snakes typically discharge malodorous secretions from these glands when provoked. Some snakes, including rattlesnakes (Crotalus spp.) and some other crotalines, defensively spray scent gland fluids.

Scent gland secretions are widely thought to deter predators, a contention supported by observations of aversive responses by ants, ophiophagous snakes, crocodynids, and carnivores. The increased size of scent glands in females and the greater pungency of their secretion prompted Kissner et al. to suggest that females depend more heavily on these organs for antipredator defense. Greene and Mason, on the other hand, demonstrated that the secretions of female brown treesnakes inhibit male courtship, serving to reject unpreferred suitors. Scent glands also are hypothesized to produce alarm pheromones.

Oldak personally discriminated among a number of snakes on the basis of the species-peculiar odors of scent gland secretions. He attributed some distinctive odors to particular lipids fractionated by TLC. For example, a band of the pinesnake (Pituophis melanoleucus) eluted from the triacylglycerol zone possessed an odor identical to that of the raw secretion. Oldak’s or other TLC studies suggest that scent gland lipids include hydrocarbons, FFAs, methyl esters, wax esters, sterols and their esters, phospholipids, and mono-, di- and triacylglycerols. Taxonomic variation in secretion composition have been described. Tolson’s analysis of the scent gland lipids of West Indian boids of the genus Epicrates revealed TLC components in insular populations of Antillean species that are absent in a continental congener, the rainbow boa (Epicrates cenchria). Tolson suggested that Antillean snakes evolved new glandular compounds for defense as they dispersed and encountered new predators.

C12–C26 FFAs and/or 6 are known from the scent glands of boid, elapid, leptotyphlopoid, and viperid snakes.
FFAs in the secretions of the Texas blindsnake (Leptotyphlops dulcis) may deter attacks by ants when it enters ant colonies to feed. C16 and C18 FFAs usually predominate in scent gland secretions. However, C20–C22 compounds are the most abundant acids observed in two crotaline snakes, the mamushi (Gloydius blomhoffii) and the western diamondback rattlesnake (Crotalus atrox).

Alkylglycerol monoethers were identified in the secretions of the western diamondback rattlesnake. Males and females of this species possess 1-O-monoalkyglycerols with C12–C20 side chains, the most abundant being 1-O-hexadecylglycerol. A TLC analysis of the eastern diamondback rattlesnake (Crotalus adamanteus) and the Florida water moccasin (Agkistrodon piscivorus conanti), however, failed to indicate bands in the zone where glycerol monoethers were expected.

The volatile compounds from the scent glands of boid, colubrid, crotaline, and/or elapid snakes include phenol, 3-methylbutanal, and acetic, propanoic, 2-methylpropanoic, butanoic, 2-methylbutanoic, 3-methylbutanoic, methylenzoic, phenylacetic, and 3-phenylpropanoic acids, all of which are strongly odorous. Two lactic acid occurs in Dumeril’s ground boa (Acrantophis dumerili), along with I and unknown terpenoids.

The nitrogenuous compounds from the scent glands include trimethylamine (in boids and colubrids) and 2-piperidone (in boids and vipers). Even-numbered fatty amides ranging from C16–C22 and C18–C24 occur in Dumeril’s ground boa and the western diamondback rattlesnake, respectively. Three additional compounds from the ground boa were characterized tentatively as amines.

Only about 6% of the scent gland secretions is amenable to extraction with organic solvents, suggesting that the bulk of these exudates consists of macromolecules. Studies by gel electrophoresis or gel filtration chromatography (GFC) of thirty-two snake species representing seven families demonstrated one to eight protein components per species, with molecular masses ranging from 10 kDa to 100 kDa. The Texas blindsnake possesses a glycoprotein containing glucosamine, galactosamine, and seventeen different amino acids. This glycoprotein is unusual in lacking tyrosine, a moderately reactive amino acid prone to oxidation and oxidative cross-linking.

Analyses of proteins in the secretions, as suggested by some investigators for scent gland lipids, may furnish information relevant to snake systematics. Weldon and Leto, for example, noted a 30 kDa component resolved by gel electrophoresis that appeared in the boids they examined, but not in the related pythonids. Investigations of these or other skin products may corroborate proposed phylogenetic schemes established by more rigorous molecular methods.

3.3.2 Nuchal gland. Ten species of Asian natricine snakes of the genera Balanophis, Macropisthodon, and Rhabdophis possess one or more paired secretory sacs called nuchal glands situated under the dorsal skin of the anterior trunk. These organs, unlike most integumental glands, arise embryologically from the mesoderm rather than the ectoderm. Nuchal glands discharge secretions, sometimes spraying them, when they rupture from pressure applied to overlying skin. The yamakagashi (Rhabdophis tigrinus), a species widespread in eastern Asia, occasionally exudes these fluids onto its dorsum when it assumes a peculiar arched-neck defensive posture.

The nuchal gland secretions of the yamakagashi are noxious to mammalian predators. The raw glandular exudates experimentally applied to the eyes of dogs and rabbits caused pupillary miosis and corneal detachment. In vitro toxicity studies using mammalian kidney and heart preparations demonstrated that bufadienolides in the secretions inhibit (Na+ + K+) ATPase and are positively inotropic, which are typical properties of these compounds.

A number of bufadienolides have been identified from the yamakagashi. Several compounds, including 76, 81, 92 and 93, are present in the red-necked keelback (Rhabdophis subminiatus) from Thailand, but the skin of Pryer’s keelback (Amphiesma pryleri), a Japanese natricine that lacks nuchal glands, does not contain them. Gamabufotalin (82) and other nuchal gland compounds also occur in the parotoid gland secretions of toads (Bufo spp.), which are preyed upon by yamakagashis.

Cholesterol (6), the metabolic precursor of bufadienolides, occurs in the nuchal glands of the yamakagashi. Mori and Burghardt, however, postulated that this snake acquires these compounds from toads, sequestering them from their prey for defense against their predators. Hutchinson et al. investigated this hypothesis by comparing the nuchal gland fluids of wild-caught snakes from areas in Japan inhabited by toads with fluids from snakes inhabiting Kinkazan Island, where toads are absent. All snakes except those from Kinkazan possessed bufadienolides 76–95. Laboratory studies demonstrated that hitching yamakagashis, even those from Kinkazan, rapidly and consistently accumulated bufadienolides in their nuchal glands when reared on North American toads (Bufo fowleri and B. terrestris), but not on prey (fish or frogs) that lack these compounds.

The toads contain mostly the conjugated bufadienolides in their nuchal glands when reared on North American toads (Bufo fowleri and B. terrestris), but not on prey (fish or frogs) that lack these compounds. The toads contain mostly the conjugated bufadienolides in their nuchal glands when reared on North American toads (Bufo fowleri and B. terrestris), but not on prey (fish or frogs) that lack these compounds.

The yamakagashi may sequester some bufadienolides, such as unaltered or after hydrolyzing their substituted arginine side chains, e.g., 82 from 102, 88 from 101, and 91 from 103. Most of the nuchal gland bufadienolides, however, appear to have been hydroxylated after dietary uptake, an alteration that may enhance their bioavailability and/or toxicity.

Riboflavin (vitamin B2), another presumed dietary component, occurs in the nuchal glands of the yamakagashi. Riboflavin also is known to impart a yellow hue to the skins of some boid, colubrid, and elapid snakes.

3.3.3 Nasal gland. Some colubrid snakes of the subfamily Psammophiinae, primarily African species, possess a paired gland situated lateral to the nasal cavity, opening through ducts near the external nares. Snakes spread secretions from this nasal gland over their body via "skin polishing," where they rub
their snout along their dorsal and ventral skin surfaces. Nasal gland fluids, which dry to form a lusterless film on the skin, are hypothesized to retard evaporative water loss or to contain pheromones used to scent-mark conspecifics and/or territories.

The watery nasal gland secretions of the Montpellier snake (*Malpolon monspessulanus*), an inhabitant of xeric Mediterranean habitats, contain proteins, electrolytes, and lipids, including C\textsubscript{16} and C\textsubscript{18} FFAs.
4 Cloacal gland: Rhynchocephalia

The Rhynchocephalia is represented by two extant species of tuatara, Sphenodon punctatus and Sphenodon guentheri, long-lived (>60 years), lizard-like inhabitants of more than 30 islands near New Zealand. Tuatara possess a paired gland that opens on both sides of the cloacal margin.9 This gland is believed to produce pheromones, although the involvement of chemical cues in the social interactions of tuatara is unclear.112

Methylene chloride extracts of the cloacal gland secretions of adult male and female Sphenodon punctatus were found to contain unusual triacylglycerols mainly comprised of two or three acyl groups derived from the following medium chain-length acids: octanoic (104), (Z)-4-octenoic (105), (4E,6Z)-4,6-octadienoic (106), 2,6-dimethyl-5-heptanonic (107), 2,6-dimethyl-5-heptenoic (108), 3,7-dimethyl-6-octenoic (109), (Z)-4-decenoic (110), (4Z,7Z)-4,7-decadienoic (111), 4,8-dimethyl-7-nonenoic (112), 2,6,10-trimethyl-9-undecenoic (113), and (E)-2,6,10-trimethyl-5,9-undecadienoic acids (114).113 Glycerides containing the common C16 and C18 acids also are present, but not together with 104–114.

Epithelial cells within the cloacal gland stain positively for carbohydrates likely associated with glycoproteins.9 Analyses by gel electrophoresis and matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS) of the secretions of male and female Sphenodon punctatus revealed a major glycoprotein with a molecular mass of ca. 50 kDa.114

5 Testudines

5.1 Rathke’s gland

All extant turtles except the Testudinidae (tortoises) and some emydid genera possess one or more pairs of Rathke’s gland, an oval-shaped organ situated outside the peritoneal cavity, adpressed to the internal lateral aspect of the shell.115,116 Ducts from this gland pass through bones and/or scutes and open through pores on the shell bridge or the skin of the axillary or inguinal regions.115–117 Turtles exude Rathke’s gland fluids, in some cases spraying them, when provoked.113 Rathke’s gland appears to be more active in young turtles.118,119 This organ is hypothesized to discharge predator repellents,116,120 pheromones,121 or excreted metabolites.122

An analysis of the Australian snake-necked turtle (Chelondina longicollis), the sole pleurodire investigated for Rathke’s gland lipids, revealed common saturated and unsaturated C16 and C18 acids, 109, and β-ionone (115).123 On the other hand, the North American stinkpot turtle (Sternoptychus odoratus), which is named for its malodorous secretions, was found to contain phenylacetic, 3-phenylpropanoic, 5-phenylpentanoic, and 7-phenylheptanoic acids (116), and lesser amounts of 3-methylbutanoic, hexanoic, hexadecanoic, and heptadecanoic acids.124 Phenylacetic acid occurs in the Rathke’s gland secretions of other cryptodires,125,126 but the other ω-phenylalkanoic acids have not been reported elsewhere from nature.

Eisner et al.124 tested the stinkpot turtle’s secretion as a feeding deterrent by topically treating beetle larvae with a mixture of ω-phenylalkanoic acids and offering them as food to swordtail fish (Xiphophorus helleri). This fish is only ca. 10 cm long and does not pose a threat to turtles. Swordtails were only mildly averse to the acid mixture. Eisner et al. suggested that ω-phenylalkanoic acids act as aposematic cues, denoting the distastefulness, pugnacity or other undesirable features of stinkpot turtles to potential predators.

TLC analyses of loggerhead (Caretta caretta)122 and Kemp’s ridley sea turtles (Lepidochelys kempi)125 have revealed bands in secretion extracts consistent with FFAs, triacylglycerols, methyl esters, sterols and their esters, and phospholipids. GC–MS analyses of these marine turtles and a freshwater species, the North American mud turtle (Kinosternon subrubrum),126 demonstrated the presence of short-chain diacids such as ethanedioic, butanedioic, pentanedioic, and 2-methylpropanedioic acids, the 2-ketoacids 2-oxopropanoic, 2-oxobutanoic, 2-oxo-3-hexanedicarboxylic acid, 2,3-dihydroxypropanoic, phenylacetic, and 4-hydroxyphenylacetic acids.122,125,126 In addition, methyl succinate, common C14–C20 fatty acids, the steroids 6 and 22, undecanal, and glyceraldehyde (117) are present among these species.
Lactic acid is a major constituent in the Rathke’s gland secretions,\textsuperscript{122,125,126} attaining concentrations in Kemp’s ridley sea turtles of 2.4 mg ml\textsuperscript{−1}.\textsuperscript{125} Weldon and Tanner\textsuperscript{122} postulated that this gland functions to excrete this and possibly other blood-borne metabolites. Measurements of the volumes of fluids released from juvenile marine turtles indicated that up to 2 mg of lactic acid can be expelled at once.\textsuperscript{118} The physiological significance of this observation, however, is unclear, pending studies on the rate of secretion replenishment.

The chief products of Rathke’s glands are proteins.\textsuperscript{116,123,124} In loggerhead and Kemp’s ridley sea turtles, Rathke’s gland fluids contain 20 mg ml\textsuperscript{−1} and 10 mg ml\textsuperscript{−1} of protein, respectively.\textsuperscript{127} Two protein fractions were resolved by GFC in both species. The primary component has a mass of 55 kDa and the smaller component has a mass of >100 kDa. The 55 kDa components of these two turtles are glycoproteins containing glucosamine. They exhibit similar amino acid compositions and are identical for the first 15 N-terminal residues.\textsuperscript{127} Characterizations of disulfide bonds and N-glycosylation sites of the 55 kDa glycoprotein from Kemp’s ridley sea turtle link it to an esterase/lipase family that includes catalytic (esterase) and noncatalytic (thryoglobulin) members.\textsuperscript{128}

A comparison by gel electrophoresis of the Rathke’s gland secretions of Kemp’s ridley sea turtle and the mud turtle suggested that they possess similar protein profiles.\textsuperscript{126} An analysis by MALDI-MS of the secretions of twenty-seven turtle species (13 cryptodires and 14 pleurodires) representing eight families indicated from three to eighteen components per species.\textsuperscript{129} Most species possess one or more proteins ranging from 59 kDa to 65 kDa, but they vary in components ≤ 35 kDa. In the Asian four-eyed turtle (Sacalia bealei), the largest detectable component was a 41 kDa glycoprotein. This comparative analysis demonstrates greater species variation in Rathke’s gland proteins than has previously been reported.

Further study of Kemp’s ridley sea turtle has revealed an enzyme with a mass of ≈200 kDa that catalyzes the cleavage of the γ-glutamyl bond in a variety of donor substrates and the transfer of the γ-glutamyl group to water (hydrolysis) or to acceptor substrates possessing a free amino group.\textsuperscript{130} This enzyme may produce peptides in a fashion similar to that of the mammalian γ-glutamyl transpeptidases. Its significance in the secretions is unclear.

### 5.2 Mental gland

More than twenty genera in the Emydidae, Platysternidae, and Testudinidae possess paired epidermal invaginations called mental glands that are situated in the throat region.\textsuperscript{131} Mental glands range in complexity from shallow (possibly vestigial) invaginations devoid of glandular tissue to large, multilobed secretory sacs. These glands are enlarged in male tortoises (Gopherus spp.) and actively secrete during the mating season.\textsuperscript{132,133} Adult desert tortoises (Gopherus agassizii) identify familiar conspecifics on the basis of mental gland secretions.\textsuperscript{133} Male Texas tortoises (Gopherus berlandieri) exhibit combat behavior, including head bobbing and shell ramming, in response to these exudates and to the C\textsubscript{8}–C\textsubscript{18} FFAs they contain.\textsuperscript{134} TLC also has suggested that triacylglycerols, sterols, and phospholipids occur in the secretions of Gopherus spp.\textsuperscript{132}

Gel electrophoresis of the glandular exudates of male and female desert, Texas, Bolson’s (Gopherus flavomarginatus), and gopher (Gopherus polyphemus) tortoises has revealed species and sexual differences in protein composition.\textsuperscript{135} Males of the closely related desert and Bolson’s tortoises displayed a band that was absent in the secretions of other males, and only male desert and Texas tortoises displayed bands denoting esterase activity. Females of all species displayed a band that was absent in males. An analysis by gel electrophoresis of male desert tortoises from Nevada, USA, revealed twelve to seventeen mental gland proteins ranging in mass from 25 kDa to 115 kDa.\textsuperscript{133} Banding patterns among males were similar, but individual differences were observed in the number and size of high mass components.

### 6 Crocodylia

#### 6.1 Gular gland

All modern crocodylians possess a paired evertible gland known as the gular gland that is located on the ventral aspect of the lower jaw in skin folds next to each mandibular ramus.\textsuperscript{135,136} Females are believed to scent mark nest sites with this gland by rubbing their lower jaw on the ground.\textsuperscript{137}

The gular gland secretions of the American alligator (Alligator mississippiensis) contain 1, 6, C\textsubscript{14}–C\textsubscript{18} FFAs, and 72.\textsuperscript{138} TLC analyses of the American alligator and other crocodylians have suggested the presence of additional compound classes, such as alcohols and triacylglycerols, in addition to demonstrating taxonomic, sexual, and individual variation in secretion composition.\textsuperscript{135,139,140}

#### 6.2 Paracloacal gland

The paracloacal gland is a paired organ embedded in the cloacal walls on each side of the vent of all modern crocodylians.\textsuperscript{136} This gland is thought to produce pheromones used in mating and/or nesting activities, but its specific function is unknown.\textsuperscript{137} We observed a small group of free-ranging juvenile American alligators in Louisiana, USA, rapidly disperse when thawed paracloacal gland secretions from several adult males were poured into a water channel where they had aggregated to feed.\textsuperscript{141} Thus, perhaps, these secretions denote aggressive adults. TLC analyses of the secretions suggest the presence of hydrocarbons, FFAs, alcohols, triacylglycerols, and their esters, and phospholipids, as well as species, sexual, and possible individual variation in secretion composition.\textsuperscript{139,140}

β-Farnesene (126) and 1 occur in all genera of caimans, \textit{Caiman},\textsuperscript{136,142,143} \textit{Melanosuchus},\textsuperscript{136} and \textit{Peleosuchus}.\textsuperscript{144} Squalene (1) comprises >50% of the secretions of adult female American crocodiles (\textit{Crocodylus acutus}), but <4% of the secretions of juveniles or adult males.\textsuperscript{145} Pentadecane is a minor component of juvenile female American crocodiles.\textsuperscript{145}

β-Springene (129), a diterpene homolog of 126, is abundant in the secretions of juvenile American alligators, but it is absent in adults.\textsuperscript{146,147} Similarly, the cyclic diterpene cembrane A (130) is
a major component (ca. 40%) of juvenile Chinese alligators (Alligator sinensis), but its concentration is reduced in the secretions of adults.\textsuperscript{147,149} Both 129 and 130 also occur in adult and immature Paleosuchus spp.\textsuperscript{144,147} An oxidized derivative of 130, 11,12-dihydrocembren-10-one (131), is unique to the Chinese alligator, where it occurs chiefly in males.\textsuperscript{147,149,150} Both 130 and 131 are the only cembrenoids known from vertebrates.

A group of related mono- and sesquiterpene hydrocarbons, each exhibiting an unusual trisubstituted conjugated diene system, was identified in the Chinese alligator and Paleosuchus spp.: (2E,4E)- and (2Z,4E)-3,7-dimethyl-2,4-octadiene (119 and 120), (2E,4E,7S)- and (2Z,4E,7S)-3,7,11-trimethyl-2,4-dodecadiene (122 and 123), and (2E,4E)- and (2Z,4E)-3,7,11-trimethyldodeca-2,4,10-dodecatriene (124 and 125).\textsuperscript{147} Some compounds were observed in individuals maintained outdoors in Florida, USA, but not in those kept at indoor facilities, thus raising the suspicion that captive conditions influence secretion composition. Myrcene (118), a monoterpenes common among plants, was observed in Paleosuchus spp.\textsuperscript{147}

Cholesterol (6) was found in secretions of the genera Alligator,\textsuperscript{148,150} Caiman,\textsuperscript{142,151} Crocodylus,\textsuperscript{145} and Osteolaemus,\textsuperscript{152} while 13 occurs only in the American crocodile.\textsuperscript{145} An early study of the paracoanal gland secretions of Caiman spp. indicated the presence of 9.\textsuperscript{153} More recently, cholesteryl formate (10) was reported in the American crocodile.\textsuperscript{145}

Compounds consisting of cholesterol or cholestanol esterified to unique \(\omega\)-phenyl C\textsubscript{20} or C\textsubscript{22} acids comprise >90% of the secretions of the African dwarf crocodile (Osteolaemus tetraspis).\textsuperscript{154} Compounds 133–140 are believed to be connected to the biosynthesis of a ketone uniquely found in this species, 3,7-diethyl-9-phenyl-2-nonanone (dianeackerone, 132).\textsuperscript{152} Yang et al.\textsuperscript{154} posited that 132 is formed by the hydrolysis of \(\beta\)-keto esters 133 and 134.

The direct analysis of secretion extracts in several studies has revealed the presence of C\textsubscript{14–C18} FFAs in alligatorids (Alligator\textsuperscript{148,150} and Paleosuchus\textsuperscript{155}) and crocodylids (Crocodylus\textsuperscript{145} and Osteolaemus\textsuperscript{152}). On the other hand, an analysis of the American alligator in which FFAs were first converted to methyl esters showed the presence of twelve unbranched acids containing seven to eighteen carbons.\textsuperscript{146} Trace amounts of a sesquiterpene acid, 3,7,11-trimethyldecaneoic acid (127), also were observed.

The presence of alcohols in the secretions of adult American alligators was suggested by TLC analyses,\textsuperscript{139,140} but GC–MS studies have revealed only 1-dodecanol and 1-tetradecanol, each present in trace amounts, exclusively in juveniles.\textsuperscript{148} Likewise, a majority of the C\textsubscript{13–C18} primary alcohols in the American crocodile were observed exclusively in hatchlings and juveniles.\textsuperscript{145} Adult Chinese alligators, on the other hand, possess an abundance of saturated and mono- and diunsaturated C\textsubscript{12–C18} primary alcohols,\textsuperscript{150} 3-Dodecen-1-ol, 1-dodecanol, and an array of unsaturated C\textsubscript{15–C18} alcohols are present in females, but absent in males. Citronellol (121) occurs in the genera Caiman\textsuperscript{142,143} and Paleosuchus,\textsuperscript{155} and 2,3-dihydrofarnesol (DHF, 128) occurs in trace amounts in the brown caiman (Caiman crocodiloides fuscus).\textsuperscript{143}

Wax monoesters are the predominant constituents of the paracoanal glands of many crocodylians. American alligators possess various esters of primary alcohols including C\textsubscript{10–C18} acetates, C\textsubscript{10–C14} butanoates, C\textsubscript{5–C18} 3-methylbutanoates, C\textsubscript{12–C14} hexanoates, C\textsubscript{12} and C\textsubscript{14} octanoates, dodecyl decanoate, C\textsubscript{10–C14} dodecanoates, C\textsubscript{10–C16} tetradecanoates, tetradeyl hexadecanoate, and dodecyl octadecanoate.\textsuperscript{146,148} With the exception of farnesyl acetate, 3-methylbutanoate, and hexanoate, the alcohol moieties of these compounds are unbranched. Sexual differences have not been observed in the composition of the
paracloacal gland secretions of American alligators, but age-class differences have been found. Aside from 129 and the C_{12} and C_{14} alcohols mentioned above, juvenile alligators uniquely possess a bishomologous series of alkanoates ranging from C_{4} to C_{18} and a series of C_{8}–C_{18} 3-methylbutanoates. Adults, on the other hand, uniquely possess certain C_{10}–C_{18} acetates and 72.

The Chinese alligator, in contrast to its American congener, chiefly contains hexadecanoates and exhibits sexual differences.
in wax ester composition. The primary alcohol moieties of these compounds range in females from C\textsubscript{7}–C\textsubscript{18} and in males from C\textsubscript{12}–C\textsubscript{18}. Hexadecenoates occur in females, but not in males. Females possess twenty-seven C\textsubscript{12}–C\textsubscript{18} acetates, whereas males possess only three monounsaturated C\textsubscript{15}, C\textsubscript{16}, and C\textsubscript{17} acetates. Alkanoates ranging from C\textsubscript{12}–C\textsubscript{18} have been found in both sexes of the Chinese alligator, but the structural details of these compounds are unknown. The chain lengths and relative abundances of the alcohol moieties of esters in the secretions of some crocodylians correspond to those of the free alcohols present, suggesting that they are precursors or, more likely, degradation products of the esters.

The paracloacal glands of the American crocodile were found to contain C\textsubscript{12}–C\textsubscript{20} acetates, C\textsubscript{13}–C\textsubscript{18} butanoates, C\textsubscript{15}–C\textsubscript{16} hexanoates, and C\textsubscript{8} and C\textsubscript{12} octadecenoates, in addition to C\textsubscript{12}–C\textsubscript{20} formates, primarily C\textsubscript{13}–C\textsubscript{18} compounds. Similar formates are known from the skin glands of some African antelopes (Bovidae, Mammalia). Citronellyl esters and a DHF ester were observed primarily in hatchling and juvenile American crocodiles. Brown caimans from Costa Rica were found to contain citronellyl acetate, butanoate, and hexanoate as well as twenty C\textsubscript{2}–C\textsubscript{18} DHF esters. DFH hexanoate and DHF octanoate also occur in the yacare caiman (\textit{Caiman yacare}).

Adults of the African dwarf crocodile, on the other hand, contain the unusual ethyl-branched ketone \textbf{132} as the major volatile component of the paracloacal glands; it is absent in juveniles. Two enantiomers of \textbf{132}, the (3\textit{S},7\textit{S})- and the (3\textit{S},7\textit{R})-isomers, were observed in proportions ranging from \textit{>9} : \textit{1} to \textit{<1} : 9, respectively. This extreme individual variation may have been introduced by including samples of different (unidentified) subspecies or secretion donors that ranged in age from 8 to 35 years. A new family of aliphatic aldehydes, ketones, and \(\beta\)-diketones possessing an ethyl branch adjacent to the carbonyl group was reported from the paracloacal glands of the common caiman (\textit{Caiman crocodilus}), the broad-snouted caiman (\textit{Caiman latirostris}), the yacare caiman, the dwarf caiman, and the smooth-fronted caiman. Forty-three compounds were observed in the \textit{Caiman} species and sixteen were observed in the \textit{Paleosuchus} species. 3-Ethylnonan-4-one (\textbf{144}), 5-ethylundecan-6-one (\textbf{155}), 2-ethyltetradecanal (\textbf{159}), 5-ethylnon-2-en-4-one (\textbf{162}), 3-ethylnon-5-en-4-one (\textbf{167}), 7-ethylnonane-4,6-dione (\textbf{180}) occur in all five species. Minor differences between males and females were noted for some compounds, but sexual variation could not be assessed because sample sizes were small and secretions were pooled from up to five adults. One unbranched ketone, heptan-3-one, was observed in a male broad-snouted caiman.

The ethyl-branched structures are not obviously related to those of other known compounds from the paracloacal glands of caimans or other crocodylians except \textbf{132}. Krückert \textit{et al.} postulated that these compounds are derived \textit{via} fatty acid- or polyketide-derived biosynthesis in which the normal malonate extender unit is replaced by an ethyl malonate unit. Such products are typical of microorganisms. Krückert \textit{et al.} observed several types of bacteria, including clostridia and propionibacteria, in the glandular secretions of a common caiman. However, they failed to isolate microbial strains producing the ethyl-branched compounds, leaving the biosynthetic origin of these compounds an open question.

7 Discussion

7.1 From TLC to structural identifications

TLC, despite its limitations compared to modern analytical methods, has revealed the complexity of lipid mixtures from the tetrapod integument and permitted tentative characterizations of
secretion components.\textsuperscript{158} TLC analyses also have indicated possible sources of variation and adaptive patterns in skin chemical profiles. Comparisons of the epidermal lipids of squamates, birds, and mammals, for example, reveal uniformity in the polar compounds, presumably due to selection for stability among components that impede water loss.\textsuperscript{3,16,159} Nonpolar lipids may contribute to the transepidermal water barrier,\textsuperscript{2,14,15} but they appear to function foremost as semiochemicals.\textsuperscript{16} TLC analyses of nonpolar lipids from the reptilian integument demonstrate taxonomic, sexual, age-class, and other sources of variation consistent with the hypothesized multifunctionality of these compounds.

TLC studies also have demonstrated anatomical differences in skin chemistry. The contrasting profiles of lipids from the epidermis and the scent glands of snakes, for example, are believed to reflect the different functions of these tissues – the epidermis as an impediment to water loss, and the scent glands as a source of predator deterrents or other semiochemicals.\textsuperscript{18,19} Detailed structural studies are needed to further elucidate contrasts in epidermal and glandular products. Lipids on the epidermis are vulnerable to evaporative loss, thus the compounds that are produced or survive there may be less volatile than are those from other exocrine sources. Similarly, fatty acids in the outer portion of the skin typically exhibit greater saturation than do those from internal tissues, and thus they are less prone to oxidative damage from the environment.\textsuperscript{40} Whether compounds from the epidermis generally exhibit greater degrees of saturation than do comparable glandular products needs to be examined.

Behavioral studies suggest the existence of regional anatomical differences in the chemistry of the epidermis. For example, snake pheromones\textsuperscript{4} and kairomones from ophiophagous snakes\textsuperscript{160} arise specifically from the dorsolateral skin. Detailed chemical comparisons of different regions of nonglandular epidermis may help to focus on the behavioral releasers in these and other organismal interactions.

### 7.2 Chemical diversity revealed

Identifications of lipids from the epidermis of squamates reveal a diverse chemistry based upon steroids, fatty acids, and their derivatives. Elucidations of compounds from tuatara, turtles, and crocodylians are needed for a more comprehensive picture of the skin surface chemistry of modern reptiles. Nonetheless, the presence of steroids, FFAs, triacylglycerols, and other lipid classes in amphibians attests to the antiquity of these compounds.\textsuperscript{3,16,15} The secretion of dietary compounds, sometimes with structural modification, may contribute to the chemical diversity of the vertebrate integument. New Guinean birds, specifically pitohuis (\textit{Pitohui} spp.) and the blue-capped ifrita (\textit{Irita kowaldi}), are prime subjects of a growing case for the defensive sequestration of ingested chemicals.\textsuperscript{165} The feathers and other tissues of these birds contain batrachotoxins, a family of steroidal alkaloids that bind Na\textsuperscript{+} channels and depolarize electrogenic membranes, thus potentially deterring predators and ectoparasites. These toxins may be acquired from the melyrid beetles (\textit{Choresine} spp.) that these birds consume.\textsuperscript{166} Similarly, as described above, Asian water snakes (\textit{Rhabdophis} spp.) appear to sequester cardiotonic bufadienolides from a type of vertebrate prey, toads.

The epidermal and glandular secretions of several squamates have been shown to contain 27, 42, and other sterols that typically are produced by plants and microorganisms. These compounds may originate in the diet or perhaps are produced by enteric bacteria. Their significance on the skin is unclear. It has been postulated that they are eliminated via the integument to prevent their excessive accumulation.\textsuperscript{55}

### 7.3 Biosynthesis by associated microorganisms?

Microorganisms residing on the mammalian integument are known to contribute to the skin chemistry of their hosts.\textsuperscript{167} The microbial lipolysis of triacylglycerols on the epidermis of humans, for example, is the main source of FFAs on the skin surface.\textsuperscript{168} Bacteria occupying the reducing environments of mammalian skin glands also generate volatile compounds, including some used as semiochemicals.\textsuperscript{167} Microorganisms contributing to the characteristic skin chemical profiles of their hosts typically exist in stable communities. Several studies have attempted to characterize the resident microbiota of reptilian skin glands in light of the possible microbial contribution to glandular chemistry.\textsuperscript{39,128,169} A study of the western diamondback rattlesnake, for example, compared bacteria in the scent glands and the cloacal fluids in order to identify scent gland residents potentially responsible for generating secretion volatiles.\textsuperscript{79} Typical enteric bacteria were
isolated from both scent glands and cloacal fluids. A few bacterial species were observed only in the scent glands, but the number of isolates was small, suggesting a low survivorship in the culture media or the occurrence of transient organisms.

Bacteria in the gular and paracloacal glands of free-ranging American alligators from Louisiana, USA, were identified to evaluate whether the different lipid profiles of these organs could be related to different populations of microorganisms. Twenty-three bacterial species were identified from both glands. Most species, however, were represented by only one isolate from either gland and many appeared to be transient enteric or water-borne organisms. Thus, as in the investigation on the origin of ethyl-branched compounds in cainmans, questions on the existence in crocodylian skin glands of a resident microbiota and on the microbial contribution to glandular exudates remain unanswered.

8 Prospectus

The integument is uniquely situated at the interface of the organism and the environment, providing a first line of defense against external insults and selectively imparting synthesized or acquired chemicals to the surroundings. Some authors have focused on water conservation achieved through the trans-epidermal barrier as the prevailing factor in shaping the chemistry of the reptilian epidermis. We point to the unrelenting and pervasive imperatives of defense against microorganisms, ectoparasites, and predators, and of pheromonal communication, as additional concurrent adaptations to terrestrial life. Demonstrations of the functions of chemicals from the integument are needed to advance more explicit evolutionary hypotheses.

We have focused on natural products from selected exocrine organs of the reptilian integument and described their known or suspected activities. The themes investigated in the studies recounted here, such as the effects of environmental variables on skin lipid composition, the microevolution of pheromones, and the dietary sequestration of skin chemicals, should prompt future studies and may clarify related topics. Quay, for example, regarded the integumental glands of reptiles as histogenetically related but nonhomologous organs. Can elucidations of glandular products or genes governing their production shed light on the evolutionary affinities of these structures? Organosulfur, heterocyclic, and macrocyclic compounds, which frequently occur in the secretions of mammalian skin glands, are seldom, if ever, reported from reptiles. Does the apparent paucity of these or other compounds in the skin of reptiles reflect constraints on their biosynthetic capabilities? What can studies of reptiles and other tetrapods reveal about the skin chemistry of early amniotes? Structural elucidations coupled with molecular methodologies and a broad zoological scope may provide the answers to these and other questions on the biosynthesis, function, and evolution of natural products from the tetrapod integument.

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