

Volatile Compounds in Shoulder Gland Secretions of Male Flying Foxes, Genus *Pteropus* (Pteropodidae, Chiroptera)

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The shoulder gland secretions of captive males of the Indian flying fox (*Pteropus giganteus*), the little golden-mantled flying fox (*P. pumilus*), the island flying fox (*P. hypomelanus*), and the large flying fox (*P. vampyrus*) were examined by gas chromatography-mass spectrometry. Sixty-five compounds, including hydrocarbons, carboxylic acids, alcohols, aldehydes, ketones, esters, and amides, were identified among the four species. Many of these compounds, such as squalene, cholesterol, and C₅–C₁₆ straight- and branched-chain carboxylic acids, are typical of tetrapod epidermal products. Aldehydes, which were detected in all four *Pteropus* species, and some straight- and branched-chain ketones, which were detected in *P. hypomelanus* and *P. pumilus*, are known from other mammalian skin glands. Acetophenone, 4-acetoxyacetophenone, and 4-hydroxyacetophenone were observed in *P. pumilus*; the last compound comprised 37.1% of the total ion current. 2,3-Butanediol, a prominent component (5.2–19.3%) in the secretions of *P. giganteus*, *P. hypomelanus*, and *P. pumilus*, and C₁₀ and C₁₂ isopropyl esters and C₁₀–C₁₄ 1-methylbutyl esters, observed in *P. hypomelanus* and *P. vampyrus*, have not previously been reported from vertebrates. α -Methyl-4-methoxybenzyl alcohol and dihydro-5-phenyl-2(3H)-furanone, from *P. giganteus* and *P. pumilus*, are new natural products. 1-Chloro-3-methyl-2-butene, another new natural product, and five C₅ compounds exhibiting a similar isoprenoid structure were observed in *P. giganteus*. Striking contrasts were observed in the chemical profiles of the species we examined, with even general chemical classes differentially represented among them.

Key words: Chiroptera, Flying Foxes, Gas Chromatography-Mass Spectrometry

Introduction

The Chiroptera, the second largest order of mammals (> 1,115 species), possesses an array of integumentary glands, many of which are presumed to produce pheromones (Quay, 1970). Despite the importance of chemoreception demonstrated in the social interactions of bats (Bloss, 1999), and reports that some taxa emit distinctive scents (e.g., Studier and Lavoie, 1984), chiropteran natural products are poorly known. A gas chromatogram of the inguinal gland secretions of the fishing bat (*Noctilio leporinus*, Noctilionidae) from Mexico displayed four major peaks (Studier and Lavoie, 1984). This chromatographic profile matched that of volatiles produced by the bacterium *Staphylococcus aureus*, which was isolated from the glands. An analysis by gas chromatography-mass spectrometry (GC-MS) of the oily secre-

tions of the subaxillary region of *N. leporinus* from Puerto Rico revealed more than 370 components, including glycolipids, nonpolar lipids, and phospholipids (Brooke and Decker, 1996). Some of these compounds were identified by referral to mass spectral databases, but most were not characterized structurally.

We analyzed by GC-MS the secretions of the shoulder glands of bats of the genus *Pteropus* (Pteropodidae), the flying foxes. This genus is the most speciose of the megachiropteran bats (ca. 60 species) and is the widest ranging, extending throughout south and southeast Asia, Melanesia, Australia, and many island groups of the Pacific and Indian Oceans (Simmons, 2005). The shoulder glands of *Pteropus* are enlarged, androgen-sensitive sebaceous glands from which males typically discharge odorous fluids during the mating season,

in some cases, soaking their upper dorsal pelage (Sokolov, 1982; Martin *et al.*, 1995). Males of some species are thought to use shoulder gland secretions to mark the boundaries of harems (Nelson, 1965; Grant and Banack, 1999) or foraging areas (Brooke and Decker, 1996). The Indian flying fox (*P. giganteus*) may use shoulder gland secretions to synchronize reproductive activities (Neuweiler, 1969). Observations at the Lubee Bat Conservancy in Gainesville, Florida, over the past 15 years indicate that male *Pteropus* spp. vigorously rub the back of their neck against ropes and shelters in their enclosures during the breeding season (August to December), leading to the visible discoloration of rubbing sites (D. LeBlanc, pers. comm.)

We collected shoulder gland secretions from captive males of *P. giganteus*, which ranges from Pakistan, India, Nepal, Sikkim, Bhutan, Myanmar, Sri Lanka, and the Maldives Islands (Indian Ocean); the island flying fox (*P. hypomelanus*), which occurs on Sulawesi and on islands from the Bay of Bengal and the Malay Peninsula to New Guinea and the Solomon Islands; the little golden-mantled flying fox (*P. pumilus*), which occurs in Palmas, Balut, Tablas, and the Mindoro Islands (Philippines); and the large flying fox (*P. vampyrus*), which inhabits southern Myanmar, Thailand, Indochina, Malaysia, Borneo, Philippines, Java, Lesser Sundas, and adjacent islands. We identified 65 compounds from these secretions, including several, *e.g.*, α -methyl-4-methoxybenzyl alcohol and dihydro-5-phenyl-2(3*H*)-furanone, not previously known from nature.

Material and Methods

Animals

Bats were housed according to species in octagonal wire mesh enclosures (width 9 m, height 2.0 m) at the Lubee Bat Conservancy in Gainesville, Florida. Each enclosure contained a temperature-controlled octagonal roost (width 3.6 m, height 2.5 m) constructed of plywood and stucco. Bats were fed daily half their weight of a mixture of apples (36%); pears (7%); peeled bananas (14%); cantaloupes (12%); carrots/sweet potatoes (9%); kale (7%); Lubee Fruit Bat Supplement (HMS Zoo Diets, Bluffton, Indiana) (6%), which contains protein, vitamins, and minerals; and seasonal fruits (9%), including apricots, grapes, kiwi

fruits, mangos, nectarines, papayas, pineapples, peaches, plums, and strawberries. Bats had access to water *ad libitum*.

Secretions were collected during August 2000 from four *P. hypomelanus* (714–826 g), three of which were born in captivity (6.5, 7.7, and 8.2 years old) and one of which was wild-caught in Indonesia in 1990, and two captive-born *P. pumilus* (210 g and 228 g; 3.4 and 5.0 years old), and in August 2004 from three captive-born *P. giganteus* (675–910 g; 6.2, 6.7, and 17.3 years old) and three *P. vampyrus* (1028–1774 g) wild caught in Indonesia in 1990 and 2000. Bats received ivermectin to control gastrointestinal parasites and rabies vaccinations (Defensor 3, Pfizer Animal Health, Exton, Pennsylvania); no other pharmaceuticals were administered to them.

Sample collection

Secretion samples were obtained by restraining bats and manually scraping a glass slide against the pelage in their nuchal region. The slides were then tipped vertically and the secretions dripped into a glass vial. Secretions were pooled according to species. Several milliliters of CH_2Cl_2 were added to each vial before being placed on dry ice.

Sample analysis

Secretion analysis was performed on the CH_2Cl_2 extracts in a splitless mode (0.5 min) using a Hewlett-Packard GCD Plus instrument fitted with a 30 m \times 0.25 mm cross-linked phenyl methyl silicone capillary column (HP-5MS). The gas chromatograph was programmed so the oven temperature was kept at 40 °C for 4 min, then increased to a final temperature of 325 °C at a rate of 30 °C/min and kept at this temperature for 2 min. Mass spectral fragments below m/z 39 were not recorded. The relative amount of each component is reported as the percent of the total ion current (TIC). Minor components less than 0.5% of the TIC were not investigated and impurities found in the solvent were subtracted from the analyses.

All compounds initially were identified by comparison of mass spectra in the NIST 1998 computerized mass spectral library, except for 1-methylbutyl dodecanoate and 1-methylbutyl tetradecanoate, which were tentatively identified by interpretation of their mass spectra. All identifications were confirmed by comparisons of

spectra and retention times to those of synthesized or purchased standards [Aldrich Chemical Co., Milwaukee, Wisconsin, and Fisher Scientific (Arcos), Pittsburgh, Pennsylvania], except for 7-methyl-3-octen-2-one, which was not commercially available.

α -Methyl-4-methoxybenzyl alcohol was prepared from 4-methoxyacetophenone by reduction with sodium borohydride in methanol. 4-Acetoxyacetophenone and 4-acetoxybenzaldehyde were prepared from 4-hydroxyacetophenone and 4-hydroxybenzaldehyde by reaction with acetyl chloride in pyridine. 3-Methyl-2-butenyl benzoate was prepared from 3-methyl-2-buten-1-ol and benzoyl chloride in pyridine. The isopropyl and/or 1-methylbutyl esters of decanoic, dodecanoic, tetradecanoic, and hexadecanoic acids were prepared by acid-catalyzed reaction of the corresponding alcohol and carboxylic acid. The mass spectra of 1-methylbutyl dodecanoate and 1-methylbutyl tetradecanoate are not in the NIST 1998 computerized mass spectral library. The EI-MS of our synthetic compounds are: $m/z = 201$ (31), 200 (21), 183 (46), 87 (19), 85 (18), 73 (22), 71 (49), 70 (100), 69 (16), 60 (33), 57 (44), 55 (48), 43 (85), 42 (19), 41 (40); and $m/z = 229$ (35), 228 (27), 211 (43), 129 (17), 87 (20), 85 (16), 73 (23), 71 (49), 70 (100), 69 (21), 60 (30), 57 (44), 55 (45), 43 (80), 41 (35); respectively. Decyl decanoate, decyl tetradecanoate, and tetradecyl tetradecanoate were prepared by acid-catalyzed reaction of the corresponding alcohol and carboxylic acid. 1-Chloro-3-methyl-2-butene was prepared from 3-methyl-2-buten-1-ol and thionyl chloride. 3-Methyl-2-buten-1-thiol was prepared from 1-chloro-3-methyl-2-butene and thiourea followed by hydrolysis with NaOH and acidification. 3-Nonen-2-one was prepared by condensation of acetone and hexanal by the method of Esafov *et al.* (1943).

The absolute stereochemistry was not determined for 2,3-butanediol, α -methyl-4-methoxybenzyl alcohol, and the 1-methylbutyl esters of decanoic, dodecanoic, and tetradecanoic acids. The diastereomers of 2,3-butanediol, the *threo* and *erythro* isomers, were not resolved by the GC-MS analysis, and samples of enantiomerically pure α -methyl-4-methoxybenzyl alcohol and the 1-methylbutyl esters of decanoic, dodecanoic, and tetradecanoic acids were not available for comparisons with secretion components.

Results and Discussion

We identified 65 compounds, including hydrocarbons, carboxylic acids, alcohols, aldehydes, ketones, esters, and amides, in the CH_2Cl_2 extracts of the four *Pteropus* species we examined (Table I). We believe that these compounds originate chiefly in the shoulder glands because the fluids we collected appeared solely in the nuchal region where the glandular field is located. We acknowledge, however, that our method of scraping the pelage to collect secretions would not exclude contaminants, such as urinary compounds that might be spread over the fur by *Pteropus* spp. during routine urine-washing (Martin *et al.*, 1995).

Many of the components that we identified, such as cholesterol (in *P. giganteus* and *P. vampyrus*) and the C_5 – C_{16} straight- and branched-chain carboxylic acids (in *P. giganteus*, *P. hypomelanus*, and *P. vampyrus*), are typical of tetrapod epidermal products. Squalene, a biosynthetic precursor of cholesterol encountered on the skin surface of some mammals (*e.g.*, Lindholm and Downing, 1980), occurs in *P. pumilus* and constitutes a major component (31.8% of TIC) of the secretions of *P. hypomelanus*.

Aldehydes, which were detected in all *Pteropus* species we examined, are known from a variety of mammalian skin glands. Benzaldehyde, which we observed in *P. giganteus*, *P. pumilus*, and *P. vampyrus*, is a major product of the anal sacs of the European pine martin (*Martes martes*) (Brinck *et al.*, 1983) and occurs in some artiodactyl cephalic glands (Flood *et al.*, 1989; Gassett *et al.*, 1997). 4-Hydroxybenzaldehyde, which also occurs in these two *Pteropus* species, is reported in the preorbital gland secretions of African antelopes (Bovidae) (Burger *et al.*, 1999), as are some of the straight- and branched-chain ketones we detected in *P. hypomelanus* and *P. pumilus* (Burger and Pretorius, 1987; Burger *et al.*, 1999).

Acetophenone, 4-acetoxyacetophenone, and 4-hydroxyacetophenone were observed in the secretions of *P. pumilus*; the last compound was especially abundant (37.1%). Acetophenone and 4-hydroxyacetophenone have been reported in castoreum, the odoriferous paste that accumulates in the paired castor (anal) sacs of beavers (*Castor* spp.) (Lederer, 1946; Rosell and Sundsdal, 2001), the temporal gland secretions of the Asian elephant (*Elephas maximus*) (Rasmussen *et al.*, 1990; Rasmussen and Perrin, 1999), and the urine of white-tailed deer (*Odocoileus virginianus*) (Miller

Table I. Major volatile compounds identified in the shoulder gland secretions of *Pteropus* spp. and their relative abundance (%).

Compound	<i>P. giganteus</i>	<i>P. hypomelanus</i>	<i>P. pumilus</i>	<i>P. vampyrus</i>
<i>Hydrocarbons</i>				
Octane	1.1			
Nonane	0.5			
Squalene		31.8	5.9	
<i>Carboxylic acids</i>				
3-Methylbutanoic acid	4.1			
3-Methyl-2-butenic acid	8.2			
Benzoic acid	12.3			8.9
Decanoic acid	3.2			
Undecanoic acid				0.8
Dodecanoic acid		2.6		3.6
Tridecanoic acid				1.9
Tetradecanoic acid	3.1	1.2		12.3
Pentadecanoic acid				1.9
9-Hexadecenoic acid				0.8
Hexadecanoic acid				2.6
9-Heptadecenoic acid				0.8
<i>Alcohols</i>				
3-Methyl-3-buten-1-ol	0.5			
3-Methyl-2-buten-1-ol	2.0			
2,3-Butanediol	5.2	12.3	19.3	
2-Heptanol		1.3		
α -Methyl-4-methoxy-benzyl alcohol	4.0			0.5
Cholesterol	1.3			0.9
<i>Aldehydes</i>				
2-Hexenal				0.5
Heptanal	0.5	0.9		0.5
2-Heptenal				0.5
Benzaldehyde	1.1		1.8	1.5
Octanal				0.5
Phenylacetaldehyde		1.6		
2-Octenal				0.5
Nonanal	2.5			0.5
2-Nonenal				0.5
2-Decenal	1.3			0.6
4-Acetoxybenzaldehyde		1.0		
4-Hydroxybenzaldehyde		2.4		2.3
<i>Ketones</i>				
2-Hexanone		1.4		
2-Heptanone		22	0.6	
3-Methyl-2-heptanone			0.7	
2-Octanone			0.6	
7-Methyl-3-octen-2-one		1.5		
Acetophenone			2.7	
3-Nonen-2-one		0.5		
2-Nonanone			10.5	
4-Phenyl-2-butanone			5.1	
2-Undecanone			2.1	
4-Acetoxyacetophenone			6.8	
4-Hydroxyacetophenone	6.7		37.1	
4-(4-Hydroxyphenyl)-2-butanone			2.6	

Table I. (cont.)

Compound	<i>P. giganteus</i>	<i>P. hypomelanus</i>	<i>P. pumilus</i>	<i>P. vampyrus</i>
<i>Esters</i>				
Isopropyl decanoate	1.1			2.4
3-Methyl-2-butenyl benzoate				0.5
Dihydro-5-phenyl-2(3 <i>H</i>)-furanone	37.5		2.7	
1-Methylbutyl decanoate				0.5
Isopropyl dodecanoate		2.2		5.8
1-Methylbutyl dodecanoate				0.8
Isopropyl tetradecanoate		2.2		3.8
1-Methylbutyl tetradecanoate				0.7
Isopropyl hexadecanoate		2.3		
Decyl decanoate				3.0
Decyl tetradecanoate				4.8
Tetradecyl tetradecanoate				8.6
<i>Amides</i>				
Urea				2.3
2,5-Pyrrolidinedione		1.9		
Benzamide		0.7		
Niacinamide		3.3	1.5	
<i>Other compounds</i>				
1-Chloro-3-methyl-2-butene	1.4			
3-Methyl-2-butene-1-thiol	0.5			
4-Vinylanisole	3.0			0.5
Unidentified		5.8		23.4

et al., 1998). The compounds in castoreum are thought to be derived from ingested plants (Ledrer, 1946).

Several of the compounds we identified from *Pteropus* spp. have not previously been reported from vertebrates. 2,3-Butanediol, for example, is a prominent component (5.2–19.3%) in the secretions of *P. giganteus*, *P. hypomelanus*, and *P. pumilus*. C₁₄ and/or C₁₆ isopropyl esters are reported from the secretions of the interdigital glands and the preorbital glands of some African antelopes (Burger, 2005), but the C₁₀ and C₁₂ isopropyl esters and C₁₀, C₁₂, and C₁₄ 1-methylbutyl esters observed in *P. hypomelanus* and *P. vampyrus* have not previously been reported from vertebrates. 1-Methylbutyl tetradecanoate has not previously been reported in the chemical literature.

Additional new natural products identified by us in the shoulder gland secretions are α -methyl-4-methoxybenzyl alcohol, present in minor quantities in *P. giganteus* and *P. vampyrus*, and dihydro-5-phenyl-2(3*H*)-furanone, present as a minor component in *P. pumilus*, but abundant (37.5%) in *P. giganteus*. We observed 1-chloro-3-methyl-2-bu-

tene in the secretion extracts of *P. giganteus*. We failed to detect this compound in the extraction solvents or in the extracts of the other bat species we examined. We also observed this compound in the extracts of several male *P. giganteus* examined in our preliminary study of this species during 2000. Halogenated compounds are rarely documented in vertebrate skin secretions. Other minor compounds present in *P. giganteus* – 3-methyl-3-buten-1-ol, 3-methyl-2-buten-1-ol, 3-methyl-2-buten-1-thiol, 3-methylbutanoic acid, and 3-methyl-2-butenic acid – possess similar isoprenoid structures and may be metabolically related to 1-chloro-3-methyl-2-butene.

Finally, we note striking contrasts in the chemical profiles of the taxa we examined. None of the 65 compounds identified from the pooled-species samples we analyzed occur in all four *Pteropus* species, and only a few, such as 2,3-butanediol, are shared among three species. Even some general chemical classes are differentially represented. Straight- and branched-chain ketones, for example, are observed only in *P. hypomelanus* and *P. vampyrus*. We suspend speculation on the signif-

inance of these apparent differences, pending investigations of the phylogenetic relatedness of the species in our study and of other factors potentially contributing to secretion composition.

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