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Volatile antimicrobial compounds in the pelage of the Mexican free-tailed bat, *Tadarida brasiliensis mexicana*

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Abstract

Gas chromatography–mass spectrometry (GC–MS) showed that CH_2Cl_2 extracts of the pelage from the Mexican free-tailed bats, *Tadarida brasiliensis mexicana*, contained only seven volatile compounds. They include two hydrocarbons, 1-octene and octane; three aldehydes, heptanal, octanal and nonanal; a carboxylic acid, nonanoic acid; and urea. It is likely that nonanal, at an average concentration 0.62 μg/mg hair, and the two homologues, heptanal and octanal found at 0.059 and 0.066 μg/mg hair, respectively, function as antimicrobial agents against mammalian skin bacteria and fungi. These three aldehydes may also act as a chemical protection against bat ectoparasites.

Keywords: Chiroptera; Scent; Antimicrobial activity; Anti-ectoparasitic agents; Short-chain aldehydes; Urine-washing

1. Introduction

There have been few studies of skin secretions produced by any chiropteran species. Recently, Wood et al. (2005) identified a composite of 65 compounds in the shoulder glands from four species of flying foxes (*Pteropus* spp.). There have been only two other investigations on the chemicals from bat skin, in which, the scent secretions of the fishing bat, *Noctilio leporinus* has been investigated (Brooke and Decker, 1996; Studier and Lavone, 1984). Most compounds in the *N. leporinus* studies were not structurally characterized and only a few were tentatively identified by referral to mass spectral databases. We report the identification of the seven volatile compounds in CH_2Cl_2 extracts from the pelage of the Mexican free-tailed bat, *Tadarida brasiliensis mexicana*, and postulate some of these compounds function as antimicrobial agents against mammalian skin bacteria and fungi. These compounds may also act as a chemical protection against bat ectoparasites.

T. brasiliensis has nine recognized subspecies and one of the most extensive distributions in the Western Hemisphere of any mammalian species (Wilkins, 1989). In North America it is found as far north as Southern Oregon and throughout the southern half of the United States, Mexico, Central America and much of the Caribbean. In South America it extends down the Pacific coast in the Andes to southern Chile and Argentina, as well as, Paraguay, Uruguay

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and the southern coastal provinces of Brazil on the Atlantic Ocean (Wilkins, 1989). Colony size for this species ranges from several dozen up to several million individuals. These bats are noted for having ''musk-like'' scent and at certain times of the year the male scent is said to be ''sweeter'' than that of the female (Herreid, 1960).

2. Material and methods

Fifteen bats, eight males and seven females, were captured after they had inadvertently entered and become trapped in the Humboldt State University Library (Humboldt County, California) during September and October 2005. Pelage samples were obtained by manually restraining each bat and excising approximately 10 mg of hair from each animal. The hair was immediately placed in individual vials; the weight determined, and then extracted by addition of 1.0 ml of CH_2Cl_2.

Gas Chromatography–mass spectral (GC–MS) analysis of the extracts was performed in a splitless mode (0.5 min) using a Hewlett–Packard G 1800 C fitted with a 30 m × 0.25 mm cross-linked phenyl methyl silicone capillary column (HP-5MS). The gas chromatograph was programmed so that 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 and impurities found in solvent control analyses were not reported.

All compounds initially were identified by comparison of mass spectra in the NIST 1998 computerized mass spectral library and confirmed by comparisons of spectra and retention times to purchased standards [Aldrich Chemical Co., Milwaukee, Wisconsin, and Fisher Scientific (Arcos), Pittsburgh, Pennsylvania]. The amount of each component per mg of hair was determined by comparison with peak areas from authentic standards.

3. Results and discussion

Only seven volatile compounds were detected in the CH_2Cl_2 extracts of the pelage from *T. brasiliensis* (Table 1). These extracts contained two hydrocarbons, 1-octene and octane; three aldehydes, heptanal, octanal and nonanal; a carboxylic acid, nonanoic acid; and urea. No notable difference was observed between males and females in the amounts of any of these compounds. The major compound in the extracts was urea, which is most likely deposited during urine-washing. Nonanal had the second highest concentration averaging 0.62 ± 0.21 μg/mg of hair (=620 ppm). The average concentration of heptanal was 0.059 ± 0.027 μg/mg of hair (=59 ppm) and for octanal it was 0.066 ± 0.027 μg/mg (=66 ppm). The carboxylic acid found in the extracts, nonanoic acid, likely arises by air oxidation of nonanal and was only observed from 11 of the 15 individuals examined.

It is likely that nonanal and the two homologues, heptanal and octanal, act as antimicrobial agents. *Trichophyton mentagrophytes*, a widely distributed zoophilic skin fungus responsible for athlete's foot and many other human skin infections, is inhibited by nonanal at a minimum inhibitory concentration (MIC) of 100 ppm (Kubo et al., 1995).

The related fungus, *Trichophyton rubrum*, has been reported to be inhibited at 280 ppm by octanal and at 154 ppm by nonanal (Kurita et al., 1981). Also, nonanal has a MIC of 100 ppm with, *Pityrosporum ovale*, a lipophilic yeast found on animal skin and implicated in human dandruff (Kubo et al., 1995). The concentration of nonanal on bat hair ranged from 300 to 900 ppm (0.30–0.90 μg/mg, Table 1), several times more concentrated than the MICs reported to inhibit *T. mentagrophytes*, *T. rubrum* or *P. ovale.*

Table 1
Volatile compounds detected in the CH_2Cl_2 extracts of *T. brasiliensis* hair

Compound	Ret. time (min.)	Frequency	Range (μg/mg hair)	Average ± SD (μg/mg hair)
1-Octene	4.83	15/15	0.007–0.019	0.012 ± 0.003
Octane	4.95	15/15	0.04–0.16	0.094 ± 0.035
Heptanal	6.19	15/15	0.02–0.11	0.059 ± 0.027
Octanal	7.07	15/15	0.03–0.13	0.066 ± 0.027
Nonanal	7.74	15/15	0.30–0.90	0.62 ± 0.21
Urea	8.32	15/15	2.3–13.6	7.7 ± 2.9
Nonanoic acid	8.61	11/15	0.0–0.16	0.070 ± 0.062

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The growth of two ubiquitous species of mammalian skin bacteria has been reported to be inhibited by two of the aldehydes found in the bat pelage. Nonanal is reported to have a MIC of 200 ppm against *Staphylococcus aureus* (Kubo et al., 1995). *S. aureus* has also been reported to be inhibited by octanal a minor component in the hair extracts (Inouye et al., 1983; Morris et al., 1979). *Propionibacterium acnes*, the human acne-causing bacterium, which is found on many other mammals, is inhibited by nonanal at a MIC of 100 ppm (Kubo et al., 1995). The MIC reported for these two pathogens is below the lowest concentration of nonanal (300 ppm) found on bat hair.

These three aldehydes may also function as a chemical protection against bat ectoparasites, since these compounds have been postulated as a seabird chemical defense against parasitic arthropods. Heptanal and nonanal are the major volatile aldehydes identified in the plumage of the whiskered auklet, *Aethia pygmaea*, a seabird found in the north Pacific and Bering Sea (Douglass et al., 2004). Octanal was the major volatile compound in the feathers of the closely related crested auklet, *Aethia cristatella* (Douglass et al., 2001, 2004). These aldehydes have been shown to be repellents of mosquitoes (Douglass et al., 2005a) and ticks (Douglass et al., 2004) but were not toxic to bird lice at the concentration found on the auklets (Douglass et al., 2005b). The role of these aldehydes and other naturally occurring compounds as ectoparasite repellents is the subject of a recent review (Bernier et al., 2007).

The source of the compounds from bat hair was not determined. The most probable origin of these compounds other than urea is the two gular glands located in the dermis of the suprasternal region. Other possible sources might be the facial sebaceous glands or salivary glands (Werner et al., 1950) or from urine-washing. The three aldehydes have previously been reported as minor component in the shoulder gland secretion of the flying fox, *Pteropus vampyrus* and octane from the flying fox, *Pteropus giganteus* (Wood et al., 2005). These aldehydes and octane have also previously been identified in the pelage of the reticulated giraffe (Wood and Weldon, 2002).

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