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VOLATILE COMPONENTS IN SCENT GLAND SECRETIONS OF GARTER SNAKES (*Thamnophis* spp.)

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Abstract—Previous analyses of the scent gland secretions of snakes have focused on the nonvolatile components. Gas chromatography–mass spectrometry of secretions from North American garter snakes (*Thamnophis butleri, T. couchi, T. elegans, T. melanogaster*, and *T. sirtalis*) indicated the following seven major volatile components: acetic, propanoic, 2-methylpropanoic, butanoic, and 3-methylbutanoic acids, trimethylamine, and 2-piperidone. Five or more of these compounds were observed in secretions of select boid, colubrid, pythonid, and viperid snakes, suggesting that they are widespread scent gland products. 3-Methylbutanal also was detected in some snake species.

Key Words—Garter snakes, *Thamnophis*, 2-piperidone, trimethylamine, short-chain carboxylic acids.

INTRODUCTION

The foul-smelling fluids discharged from the cloacal region of snakes are familiar to anyone who has handled an agitated specimen. Contributing to these odoriferous materials are the scent glands, two organs opening through separate ducts at the margin of the cloacal orifice (Whiting, 1969). Previous analyses of scent gland secretions have focused on the nonvolatile components. Both lipids (Oldak, 1976; Weldon et al., 1990, 1991, 1992; Simpson et al., 1993) and

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proteins (Weldon and Leto, 1995) have been reported. Studies on the volatile components of scent gland secretions are confined to an analysis of an ether extract of Drumeril's ground boa, *Acrantophis dumerili*, Boidae (Simpson et al., 1993). Short-chain carboxylic acids (C_3–C_5) and phenol, in addition to higher molecular weight compounds, were identified in this species' secretions.

We report here an analysis by gas chromatography–mass spectrometry (GC-MS) of the volatile components in the scent gland secretions of North American garter snakes (*Thamnophis* spp.). This is the first detailed identification of scent gland compounds from the Colubridae, the most speciose family of snakes. In addition, we provide preliminary results on the volatile secretions of select boid, colubrid, pythonid, and viperid snakes. Our results point to some compounds that likely are widespread scent gland products.

METHODS AND MATERIALS

Scent gland secretions were obtained from adult free-ranging and captive snakes. Free-ranging specimens of the western aquatic garter snake, *T. couchi* (one female), wandering garter snake, *T. elegans* (one female), and eastern garter snake, *T. sirtalis* (three females), were captured in Humboldt County, California; all individuals were released after samples were obtained. Captive secretion donors maintained in the Reptile Ethology Laboratory of the University of Tennessee, Knoxville, were *T. sirtalis* (four females, five males), captured in or born to females from Ottawa County, Ohio; the black-bellied garter snake, *T. melanogaster* (eight females, four males), captured in or born to females from Jalisco, Mexico; and Butler's garter snake, *T. butleri* (five females, three males), born to females captured in Wayne County, Michigan. Captive secretion donors from the Dallas Zoo, Texas, and the Houston Zoological Gardens, Texas, were *T. sirtalis* (one female, one male); *T. elegans* (one male); Dumeril's ground boa, *Acrantophis dumerili*, Boidae (one male); green anaconda, *Eunectes murinus*, Boidae (one female); common kingsnake, *Lampropeltis getula*, Colubridae (one male); ringed python, *Bothrochilus boa*, Pythonidae (one female); copperhead, *Agkistrodon contortrix*, Viperidae (one female); and speckled rattlesnake, *Crotalus mitchelli*, Viperidae (one female).

Scent gland secretions, expressed by gently pressing on the base of the tail, were collected in glass vials with Teflon-lined stoppers. Samples from free-ranging snakes from California were analyzed individually by GC-MS within 30 min. of collection. The pH of the secretions from these snakes was determined with pHydrion Paper (Micro Essential Laboratory, Brooklyn, New York) within a minute of collection. Samples from captive snakes were pooled by species and sex, placed on Dry Ice, shipped to California, and analyzed within a few hours of receipt. Approximately 1 μl of neat secretion was used for each GC-MS analysis.

GC-MS was done on a Hewlett-Packard gas chromatograph (model 5890) fitted with a mass selective detector (model 5970) using a 12-m cross-linked methyl silicone capillary column (HP-1). The gas chromatograph was programmed so the oven temperature was maintained at 40°C for 4 min., then increased to a final temperature of 250°C at a rate of 30°C/min and kept at this temperature for 4 min. For native secretions, mass spectral fragments below $m/z = 20$ were not recorded, and the mass spectrometer was turned on 0.1 min after injection of the sample so that all volatile components could be analyzed. For ether extracts, fragments below $m/z = 35$ were not recorded, and the mass spectrometer was turned on 2 min after injection of the sample. Compounds were identified by comparing their mass spectra and relative retention times of those of authentic standards (Aldrich Chemical Co., Milwaukee, Wisconsin).

To examine the possibility that trimethylamine was an artifact from the decomposition of nonvolatile trimethylammonium salts on the hot GC injector, 0.1 μl of 10% NaOH was added to the vial containing the secretions from *E. murinus*. The vial was immediately sealed with a rubber stopper. After 5 min, a 5-μl headspace sample was analyzed by GC-MS analysis using the same conditions as for native secretions.

To see if trimethylammonium salts of the short-chain carboxylic acids generate trimethylamine and the free carboxylic acids on injection into the GC-MS, a solution was prepared from 50 ml of water and 3 drops of each carboxylic acid. This was adjusted to pH 7 with an aqueous solution of trimethylamine and then analyzed by GC-MS using the same conditions as for the native secretions.

The presence of ammonia or low-molecular-weight amines was investigated by treating the secretions of *T. sirtalis* with 1 ml of 1.0 M NaOH and 1 drop of benzoyl chloride in 1 ml of ether. The ether solution was analyzed by GC-MS for benzamide and *N*-substituted benzamides.

To examine the possibility that 2-piperidone was an artifact from the dehydration of 5-aminopentanoic acid, 1.0 μl of a solution of 100 mg of 5-aminopentanoic acid (Aldrich Chemical Co.) in 50 ml was analyzed by GC-MS using the same conditions as for native secretions.

RESULTS AND DISCUSSION

GC-MS analysis of the neat scent gland secretions from samples of all *Thamnophis* species showed the same seven volatile compounds (in order of elution): trimethylamine, acetic acid, propanoic acid, 2-methylpropanoic acid, butanoic acid, 3-methylbutanoic acid, and 2-piperidone (Figure 1).The relative amounts of these compounds varied between samples, and no consistent variation was seen between sex or species. The electron impact-mass spectra of these compounds are as follows: trimethylamine—$m/z = 59(M^+, 42), 58(100), 44(11),$

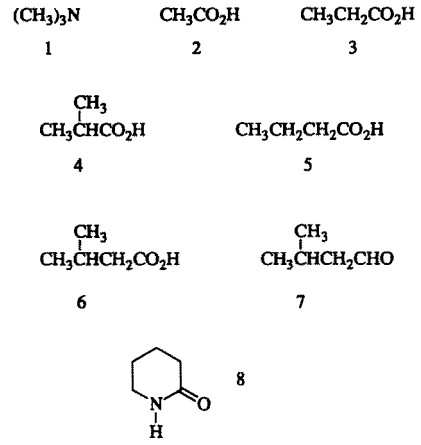

FIG. 1. Volatile components from the scent gland secretions of snakes: trimethylamine (1), acetic acid (2), propanoic acid (3), 2-methylpropanoic acid (4), butanoic acid (5), 3-methylbutanoic acid (6), 3-methylbutanal (7), and 2-piperidone (8).

43(18), 42(92), 41(25), 40(21), 30(50), 28(42), and 27(16), acetic acid—m/z = 61(1), 60(M^+, 48), 45(96), 43(100), 42(16), 41(6), 40(2), 31(7), 29(25), and 28(13), propanoic acid—m/z = 74(M^+, 16), 73(16), 57(9), 45(42), 44(11), 30(14), 29(65), 28(100), 27(68), and 26(35), 2-methylpropanoic acid—m/z = 73(20), 45(68), 44(28), 43(100), 42(21), 41(65), 39(44), 29(28), 28(81), and 27(100), butanoic acid—m/z = 73(39), 60(100), 45(38), 43(24), 42(50), 41(39), 39(32), 29(28), 28(62), and 27(56), 3-methylbutanoic acid—m/z = 87(20), 60(100), 45(68), 43(62), 42(42), 41(68), 39(68), 29(37), 28(24), and 27(62), and 2-piperidone—m/z = 99(M^+, 42), 71(30), 55(26), 44(24), 43(56), 42(74), 41(62), 39(32), 30(65), and 28(100).

The retention time of the amine and carboxylic acids varied between samples, a likely result of water in the secretions binding to the column and altering its polarity. To examine this possibility, we compared the retention times of pure acetic acid and an aqueous solution of it. Glacial acetic acid exhibited a retention time of 1.71 min. When 1 μl of a solution prepared from three drops of glacial acetic acid and 50 ml of distilled water was injected, acetic acid had a retention time of 5.16 min. The retention time of the other short-chain carboxylic acids and trimethylamine also increased when injected as aqueous solutions, but the relative order of elution remained constant. The retention time of 2-piperidone was 8.17 min for all runs.

The possibility that trimethylamine is an artifact from decomposition on the hot GC injector of trimethylammonium salts, a type of Hofmann degradation, was investigated. Trimethylamine was detected in the headspace over a

sample of secretions that was treated with a NaOH solution in a sealed container, indicating the compound is not an artifact.

The pH of the secretions from free-ranging garter snakes from Humboldt County was 7, indicating the trimethylamine and short-chain carboxylic acids exist in the ionized form. An aqueous solution of the short-chain carboxylic acids adjusted to pH 7 with trimethylamine was subjected to the same GC-MS analysis as native scent secretions. Free trimethylamine and carboxylic acids were observed; therefore, we surmise the heat of the injector or separation on the column lead to the generation of these species from trimethylammonium salts of the short-chain acids.

Low-molecular-weight amines or ammonia present with the trimethylamine might not be detectable on GC-MS analysis, so a sample of the secretions from *T. sirtalis* was treated with an ethereal solution of benzoyl chloride and an aqueous solution of sodium hydroxide (Shotten-Baumann reaction). GC-MS analysis of the resulting ether solution failed to show any benzamide or *N*-substituted benzamide derivatives; thus, these amines did not escape detection.

To examine the possibility that 2-piperidone was an artifact produced by the dehydration of 5-aminopentanoic acid, which is known to occur in some mammalian skin gland secretions (Albone *et al.*, 1976), an aqueous solution was analyzed by GC-MS, as was done with the native scent secretions. No cyclic lactam was observed; thus, 2-piperidone appears to be an actual component of the secretions.

A comparison of the scent gland secretions from other snakes demonstrates

TABLE 1. VOLATILE COMPONENTS FROM SCENT GLAND SECRETIONS OF SNAKES

	Compound[a]							
Family and species	1	2	3	4	5	6	7	8
Boidae								
Acrantophis dumerili	X	X	X	X	X	X	X	X
Eunectes murinus	X	X	X	X	X	X	ND[b]	ND
Colubridae								
Lampropeltis getula	X	X	X	X	X	X	ND	ND
Pythonidae								
Bothrochilus boa	ND	X	X	X	X	X	ND	ND
Viperidae								
Agkistrodon contortrix	ND	X	X	X	X	X	ND	X
Crotalus mitchelli	ND	X	X	X	X	X	X	X

[a]Trimethylamine (1), acetic acid (2), propanoic acid (3), 2-methylpropanoic acid (4), butanoic acid (5), 3-methylbutanoic acid (6), 3-methylbutanal (7), and 2-piperidone (8).
[b]ND = none detected.

some of the components found in *Thamnophis* (Table 1). All species were found to contain the same short-chain carboxylic acids. Trimethylamine was detected in *A. dumerili*, *E. murinus*, and *L. getula*, but not in the other snakes examined. 2-Piperidone was identified in the secretions from *A. dumerili*, *A. contortrix*, and *C. mitchelli*. *A. dumerili* and *C. mitchelli* secretions contained 3-methylbutanal, a compound not observed in the analysis of *Thamnophis* secretions. This compound had a retention time of 1.34 min and the mass spectrum m/z = 71(13), 58(25), 44(100), 43(50), 42(18), 41(71), 39(31), 29(50), 28(24), and 27(46).

Phenol and 2-methylbutanoic acid detected in a previous study of *A. dumerili* (Simpson *et al.*, 1993) were not seen on analysis of native secretions. Furthermore, the three suspected amines isolated by extraction and thin-layer chromatography of *A. dumerili* secretions were not seen in this study. Injection of neat samples of these secretions likely masks these minor components because of the high concentrations of the short-chain carboxylic acids.

The antipredator function generally attributed to scent gland secretions (e.g., Greene, 1987) needs to be examined. Trimethylammonium salts of short-chain carboxylic acids may themselves be effective defensive compounds. Furthermore, if the secretions are introduced into an alkaline environment, trimethylamine, a pungent gas with an ammonia-like odor, is released. If the secretions encounter an acidic environment, the short-chain carboxylic acid become volatile and more offensive. 2-Piperidone, the least volatile component we observed, while not itself strongly odoriferous, is bitter (tasted by W.F.W.).

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