

VOLATILE COMPONENTS IN DEFENSIVE SPRAY OF THE HOODED SKUNK, *Mephitis macroura*

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Abstract—GC-MS analysis of the anal sac secretion from the hooded skunk, *Mephitis macroura*, showed the following seven major components comprised 99% of the volatiles in this secretion: (*E*)-2-butene-1-thiol, 3-methyl-1-butanethiol, *S*-(*E*)-2-butenyl thioacetate, *S*-3-methylbutenyl thioacetate, 2-phenylethanethiol, 2-methylquinoline, and 2-quinolinemethanethiol. Minor volatile components identified in this secretion are phenylmethanethiol, *S*-phenylmethyl thioacetate, *S*-2-phenylethyl thioacetate, bis[(*E*)-2-butenyl] disulfide, (*E*)-2-butenyl 3-methylbutyl disulfide, bis(3-methylbutyl) disulfide, and *S*-2-quinolinemethyl thioacetate. This secretion is similar to that of the striped skunk, *Mephitis mephitis*, differing only in that it contains four compounds not reported from the striped skunk: phenylmethanethiol, *S*-phenylmethyl thioacetate, 2-phenylethanethiol, and *S*-2-phenylethyl thioacetate.

Key Words—Mephitidae, *Mephitis macroura*, hooded skunk, anal sac secretion, (*E*)-2-butene-1-thiol, 3-methyl-1-butanethiol, *S*-(*E*)-2-butenyl thioacetate, *S*-3-methylbutenyl thioacetate, 2-phenylethanethiol, 2-methylquinoline, 2-quinolinemethanethiol.

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INTRODUCTION

The defensive secretions sprayed from the anal sac of skunks are well known for their malodorous nature. The striped skunk has been the subject of many chemical investigations (Swarts, 1862; Löw, 1879; Aldrich, 1896; Aldrich and Jones, 1897; Stevens, 1945; Andersen and Bernstein, 1975; Andersen et al., 1982; Wood, 1990). The volatile components in the spray of two other North American skunks, the spotted skunk (Wood et al., 1991) and the hog-nosed skunk (Wood et al., 1993) have also been identified. In the present study, we report the volatile components of the anal sac from the hooded skunk, *Mephitis macroura*.

METHODS AND MATERIALS

The defensive secretion from a single male hooded skunk from Hidalgo County, New Mexico, USA, was examined. A sample of the secretion was obtained from each anal sac of the anaesthetized animal by inserting a needle into the sac and withdrawing the contents. The secretion was placed in a screw-cap vial, and shipped at ambient temperature to Arcata, California, USA. Within 3 days of collection, a 20-mg portion of this secretion from each gland was placed in 1.5 ml of dichloromethane and analyzed by gas chromatography–mass spectrometry (GC-MS).

The GC-MS analyses were performed on the dichloromethane extracts in a splitless mode (0.5 min), using a Hewlett-Packard GCD Plus 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 was determined as the percent of the total ion current (TIC).

Identification of the components was done by comparison of mass spectra and gas chromatographic retention times to reference compounds. (*E*)-2-Butene-1-thiol, *S*-(*E*)-2-butenyl thioacetate, *S*-3-methylbutenyl thioacetate, 2-quinoline-methanethiol, and *S*-2-quinolinemethyl thioacetate were obtained from striped skunk secretion (Andersen and Bernstein, 1975; Wood, 1990). 3-Methyl-1-butane-thiol, 2-phenylmethanethiol, and 2-methylquinoline were purchased from Aldrich Chemical Co. 2-Phenylethanethiol was prepared by the method of Urquhart et al. (1955) from (2-bromoethyl)benzene. *S*-phenylmethyl thioacetate and *S*-2-phenylethyl thioacetate by reacting phenylmethanethiol or 2-phenylethanethiol with acetyl chloride. The mass spectrum of *S*-phenylmethyl thioacetate is (EI-MS): $m/z = 166$ (M^+ , 19), 124(9), 123(26), 122(20), 121(5), 92 (8), 91(100), 89(5), 77(9), 64(17), 63(7), 51(9), 50(4), 45(20), and 43(72), and that of *S*-2-phenylethyl thioacetate is (EI-MS): $m/z = 180$ (M^+ , 3), 135(3), 105(12), 104(100), 103(3), 92 (5), 91(32),

79(3), 78(5), 77(9), 65(9), 63(3), 51(6), 43(44), and 39(4). Bis[(*E*)-2-butenyl] disulfide, (*E*)-2-butenyl 3-methylbutyl disulfide, and bis(3-methylbutyl) disulfide were prepared by treatment of the defensive secretion with iodine as described by Wood (1990). Mass spectra have previously been reported for (*E*)-2-butene-1-thiol, 3-methyl-1-butanethiol, *S*-(*E*)-2-butenyl thioacetate, *S*-3-methylbutenyl thioacetate, 2-methylquinoline, 2-quinolinemethanethiol, bis[(*E*)-2-butenyl] disulfide, (*E*)-2-butenyl 3-methylbutyl disulfide, and bis(3-methylbutyl) disulfide by Wood (1990); for 2-phenylethanethiol by Wood et al. (1991); and for phenylmethanethiol in the NIST 1998 computerized mass spectral library.

RESULTS AND DISCUSSION

The GC-MS analyses on two samples of hooded skunk anal sac secretion are reported in Table 1. The variation in composition between the two anal sacs was small and disappeared on rounding to two figures. The analyses showed seven major components at a concentration greater than 1% to make up 99% of the volatiles (Figure 1). These compounds and their retention times (R_t), are (*E*)-2-butene-1-thiol (**1**, $R_t = 3.06$ min), 3-methyl-1-butanethiol (**2**, $R_t = 4.19$ min),

TABLE 1. COMPOSITION OF MAJOR VOLATILE COMPONENTS OF ANAL SAC SECRETION FROM FOUR SPECIES OF NORTH AMERICAN SKUNKS^a

Compound	Amount (%)			
	Hooded skunk ^b	Striped skunk ^c	Spotted skunk	Hog-nosed skunk
(<i>E</i>)-2-Butene-1-thiol	32	38–40	30–36	71
3-Methyl-1-butanethiol	39	18–26	48–66	
<i>S</i> -(<i>E</i>)-2-Butenyl thioacetate	16	12–18		17
<i>S</i> -3-Methylbutanyl thioacetate	7	2–3		
Phenylmethanethiol	0.3		0.2–0.8	1
2-Phenylethanethiol	1.4		2–5	
2-Methylquinoline	2.3	4–11	0.3–0.9	2
Bis[(<i>E</i>)-2-butenyl] disulfide	trace		trace	3
<i>S</i> -Phenylmethyl thioacetate	trace			
(<i>E</i>)-2-Butenyl 3-methylbutyl disulfide	0.5	0.2–1.6	0.2–0.6	
Bis(3-methylbutyl) disulfide	trace		0.1–0.2	
<i>S</i> -2-Phenylethyl thioacetate	0.2			
2-Quinolinemethanethiol	1.3	4–12	0.2–0.3	0.5
<i>S</i> -2-Quinolinemethyl thioacetate	trace	1–4		

^aIn order of increasing retention times. Data from Wood (1990) and Wood et al. (1991, 1993).

^bVariation of composition for the two anal sacs was small and disappeared on rounding to two figures.

^cCompounds less than 1% were not identified in this study.

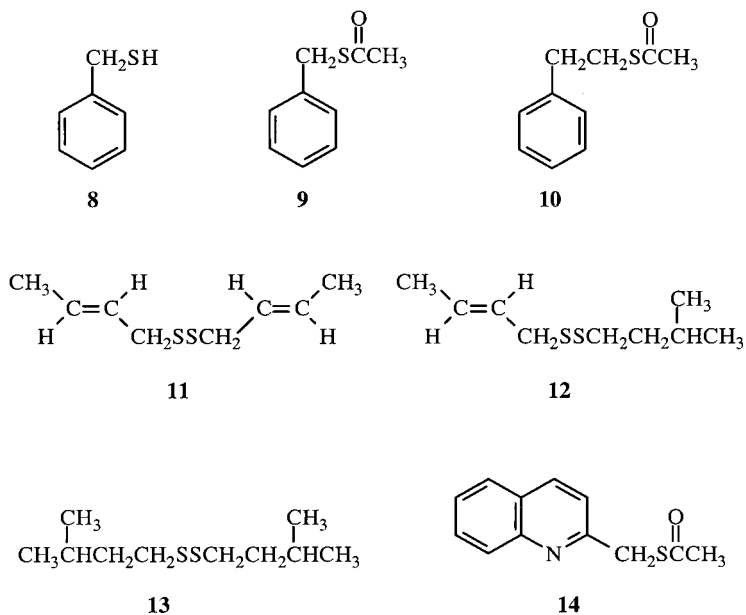


FIG. 2. Minor components identified in *Mephitis macroura* defensive secretion: phenylmethanethiol (**8**), *S*-phenylmethyl thioacetate (**9**), *S*-2-phenylethyl thioacetate (**10**), bis[*(E)*-2-butenyl] disulfide (**11**), (*E*)-2-butenyl 3-methylbutyl disulfide (**12**), bis(3-methylbutyl) disulfide (**13**), and *S*-2-quinolinemethyl thioacetate (**14**).

not from the hog-nosed skunk (Wood et al., 1993). The relative amounts of the two most abundant compounds are also different. In the hooded skunk, 3-methyl-1-butanethiol is the major compound, while (*E*)-2-butene-1-thiol is more abundant in the striped skunk.

The hooded skunk, striped skunk, and the hog-nosed skunk have a thioacetate derivative for each of the free thiols in their secretion. Perhaps the components in these skunks' defensive spray are biosynthesized as the thioacetates and then hydrolyzed to the thiol, as thioacetates are used in many biosynthetic pathways. These thioacetates may make the effects of this secretion longer lasting. The thioacetate derivatives can hydrolyze slowly and continue to release the offensive-smelling thiols over a period of time. Anecdotal reports about pets sprayed by striped skunks, indicate that skunk odor later returns to sprayed animals when their hair is wet.

Several DNA studies have been done on the relatedness of the three skunk genera: striped and hooded skunks (*Mephitis*), spotted skunks (*Spilogale*), and hog-nosed skunks (*Conepatus*). These studies indicate that skunks of the genus *Mephitis* and *Spilogale* may be more closely related to each other than either is to the genus *Conepatus* (Dragoo et al., 1993; Dragoo and Honeycutt, 1997). Variations

in the defensive spray of these three genera were examined to see if they support this relationship (Table 1). Of the major free thiols, *Mephitis* secretion has (*E*)-2-butenethiol, methylbutanethiol, and phenylethanethiol as does *Spilogale*, while *Conepatus* has only (*E*)-2-butenethiol. On this basis, *Mephitis* and *Spilogale* may seem more closely related. However, *Mephitis* and *Conepatus* have thioacetate derivatives of all of the free thiols, types of compounds that are missing from *Spilogale*. On this basis, *Mephitis* and *Conepatus* seem to be more closely related. Thus, examination of presently known secretion components can neither confirm nor refute genetic studies, but if the secretions from other *Conepatus* spp. are different, they could support these studies.

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