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PUTATIVE CHEMICAL SIGNALS FROM WHITE-TAILED DEER (Odocoileus virginianus). URINARY AND VAGINAL MUCUS VOLATILES EXCRETED BY FEMALES DURING BREEDING SEASON

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Abstract—Urine and vaginal mucus samples from female white-tailed deer in estrus and mid-cycle were analyzed by combined gas chromatography–mass spectrometry (GC-MS). Forty-four volatiles were found in mucus and 63 in urine. The volatiles common to both vaginal mucus and urine included alcohols, aldehydes, furans, ketones, alkanes, and alkenes. Aromatic hydrocarbons were present only in the vaginal mucus, whereas pyrans, amines, esters, and phenols were found only in urine. Both estrous mucus and estrous urine could be identified by the presence of specific compounds not present in mid-cycle samples. Numerous compounds exhibited dependency on ovarian hormones.

Key Words—Chemosignals, deer, *Odocoileus virginianus*, urine, vaginal mucus, volatiles.

INTRODUCTION

White-tailed deer (*Odocoileus virginianus*) have a complex scent communication system that is insufficiently understood at present. Whitetails form two basic types of social units, doe (matriarchal) groups and buck groups. Usually there

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is little or no contact between adult does and adult bucks except during the autumn breeding season (Marchinton and Hirth, 1984). Various aspects of marking behavior performed by males during the breeding season have been extensively examined (Moore and Marchinton, 1974; Miller et al., 1987a,b; Marchinton et al., 1990). White-tailed deer behavior suggests that urine could be a source of chemical signals indicating estrous status of the female (Moore and Marchinton, 1974). Female urine elicits a flehmen response from males. Additionally, Whitney et al. (1992) have presented evidence that vaginal mucus or other secretions from the reproductive tract are the source of a signal(s) indicating female reproductive status. They argue that urinalysis via vomeronasal stimulation likely affects the animal's reproductive physiology, whereas volatiles from the reproductive tract that are detected by the main olfactory system elicit approach and courtship behaviors.

Clearly, chemosignals from urine and vaginal secretion play important roles in the reproductive physiology of white-tailed deer. In this study we have investigated the urinary and vaginal mucus volatile compounds excreted by female white-tailed deer during the breeding season. Qualitative changes and concentrations of volatiles at estrus and mid-cycle as well as concentration changes in relation to the presence of ovarian hormones were investigated.

METHODS AND MATERIALS

Subject and Sample Collection. Samples of urine and vaginal mucus were collected from four adult (≥ 2 years old) female deer, housed in individual stalls (3×6 m) at the University of Georgia's Whitehall Deer Research Facility. Females were fed ad libitum a commercial feed (Omolene 300, Purina Mills, Inc.). Bermuda grass (*Cynodon dactylon*) or alfalfa (*Medicago sativa*) hay was also provided ad libitum.

Voided urine and vaginal secretions from estrous and mid-cycle females and urine from ovariectomized females were collected during the breeding season (i.e., October–January). Each female was allowed to associate daily with a mature, sexually experienced, epididymectomized male. Behavioral patterns of white-tailed deer indicative of estrus have been described by Warren et al. (1978) and Brown and Hirth (1979). Estrus was identified as the day(s) on which the male displayed courtship behaviors toward the female and the female was receptive to mounting by the male. Mid-cycle was identified as the midpoint between consecutive periods of estrus during the 26-day estrous cycle (Knox et al., 1988).

Mucus was collected by flushing the vagina/cervical area with 10 ml of sterile physiological saline. Voided urine was collected from four intact and three ovariectomized females. Studied animals were sedated using a mixture of

xylazine hydrochlorine and ketamine hydrochloride as described by Mech et al. (1985) and placed in a modified transport crate fitted with a screened bottom. Anesthesia was reversed with yohimbine hydrochloride and the females were held in the crate until urination. The mean time until urination was 3 hr 10 min. Immediately after urine and mucus collection, three 1.5-ml samples of each type were frozen and stored at −20°C until analyzed.

Procedure. Urinary and vaginal mucus volatiles were concentrated on porous polymer, Tenax GC, using the modified headspace technique developed by Novotny et al. (1974) and McConnell et al. (1979). To give each sample (1.5 ml) the same high ionic strength, 0.4 g of $(NH_4)_2SO_4$ was added. Next, the sample was absorbed on 0.6 g of glass wool. Purified helium gas at a flow of 30 ml/min was then passed through the glass wool for 30 min to purge the volatiles from it. The headspace sampling was performed at 50°C. After passing through a cooled condenser, installed to reduce water mist originating from the sample, the volatiles were trapped on a 4-mg Tenax GC precolumn packed into the injection port glass liner of a gas chromatograph.

After the headspace sampling procedure, the precolumn with trapped volatiles was inserted into the modified injection port of a Hewlett-Packard model 5980A gas chromatograph–mass spectrometer (GC-MS). The volatiles were desorbed at 200°C and retrapped cryogenically for 15 min to compress the desorbed sample at the column inlet. The column used was a glass capillary column (30 m × 0.25 mm ID) that had been statically coated with UCON-5-HB-200 (Supelco Inc., Bellefonte, Pennsylvania). Column temperature was programmed from 30 to 160°C at a rate of 2°C/min. Volatile constituents were identified through their mass spectra, using electron impact ionization at 70 eV. Peak areas were evaluated using a GC-MS data station. The identification of volatile components was verified by retention time measurements of authentic compounds.

Statistical Analysis. Statistical comparison of the concentration of excreted volatiles (expressed by integrated peak areas) was performed by using one-way analysis of variance (F at $P < 0.05$) with the Tukey test for equal sample size ($P < 0.02$). Because of the heteroscedastic data, the inequality of variance was "corrected" by logarithmic transformation of the original X values (integrated peak areas in arbitrary units) to $X' = \log X + 1$ values (Zar, 1984).

RESULTS AND DISCUSSION

Headspace analysis of the volatile compounds from the test materials revealed a large number of common compounds. Some varied considerably in concentration among samples. Others, present at constant levels, commonly

accounted for more than half of the total number of compounds observed in the samples. Only a few tended to peak consistently in relation to reproductive state and hormonal change.

Volatiles identified in both vaginal mucus and urine of female deer include alcohols, aldehydes, furans, ketones, alkanes, and alkenes. Pyrans, amines, esters, and phenols were present in the urine but not in the vaginal mucus. Conversely, aromatic hydrocarbons were characteristic of the vaginal mucus only. Several classes of volatiles that we observed in white-tailed deer have been reported from the urine of other mammals including mice, voles, and humans (Albone, 1984; Novotny et al., 1990).

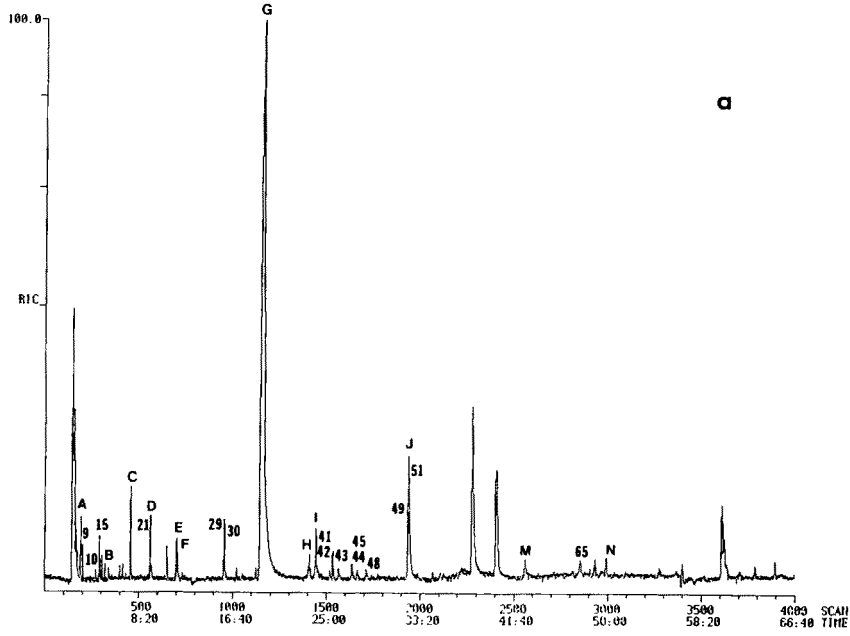

FIG. 1. GC-MS profiles of volatile compounds from (a) mucus and (b) voided urine of white-tailed deer females. (a) Not all peaks representing the identified compounds were numbered. The numbers in Tables 1 and 2 correspond to the numbers in the chromatogram. The peaks marked by letters represent compounds characteristic for estrous mucus only. The numbered peaks represent compounds common for mucus and urine. (b) Not all peaks representing the identified compounds were numbered. The numbers in Tables 3 and 4 correspond to the numbers in the chromatogram. All numbered peaks in the chromatogram represent ovary-dependent compounds. The numbered peaks marked additionally with filled dots represent compounds characteristic for estrous urine only.

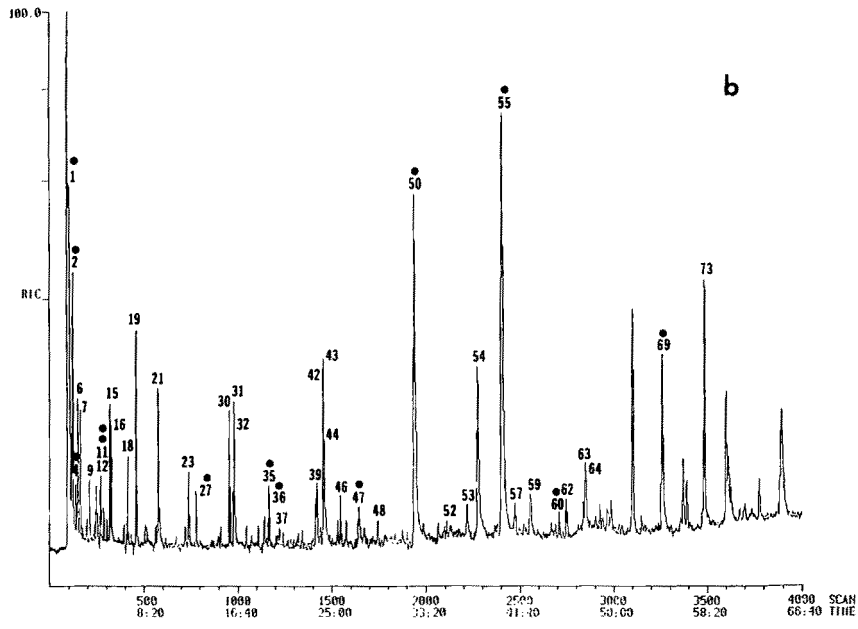

FIG. 1. Continued

The GC-MS recording shown in Figure 1a is typical of the volatile profiles obtained from the vaginal mucus of estrous females. Forty-four volatiles (Tables 1 and 2 and Table 5 below) occurred in vaginal mucus in measurable quantities; the majority of them were tentatively identified. Fourteen of these compounds, however, were found to be characteristic of the vaginal mucus only (Table 1). The composition of these characteristic compound mixtures depended upon the phase of the females' reproductive cycle. Estrous females discharged a mucus that was richer in the characteristic volatiles than that of the females in mid-cycle (Table 1). Nine of the 14 characteristic compounds (64%) were produced only by estrous females, whereas only two (14%) were produced by the females exclusively during mid-cycle. Certain aromatic hydrocarbons, whose presence may or may not be significant, along with two alcohols (2-propanol and 2-propyl-1-decanol) and two ketones (2-hexanone and methylacetophenone) occurred only in estrous vaginal samples (Table 1). Since estrous vaginal secretion, but not mid-cycle secretion, has been shown to elicit courtship behavior in male white-tailed deer (Whitney et al., 1992; Murphy et al., 1994), these compounds may represent behaviorally important chemosignals. Behavioral

TABLE 1. VOLATILE COMPOUNDS CHARACTERISTIC OF VAGINAL MUCUS

Class of compounds	Peak number	Structure	Integrated peak areas (mean ± SEM)[a]	
			Estrus	Mid-cycle
Alcohols	A	2-propanol	0.78 ± 0.04	0[b]
	B	2-butanol	1.83 ± 0.01	1.82 ± 0.01
	I	cyclohexanol	2.77 ± 0.02	2.97 ± 0.01
	K	M.W. 156	0	2.75 ± 0.01
	M	2-propyl-1-decanol	1.88 ± 0.01	0
Aromatic hydrocarbons	D	*p*-xylene	0.50 ± 0.00	0
	E	*m*-xylene	1.61 ± 0.01	0
	F	*o*-xylene	1.47 ± 0.06	0
	H	phenylacetylene	0.71 ± 0.02	0
	J	indene	1.11 ± 0.02	0
Furans	L	2-acetyl-5-ethylfuran	0	2.01 ± 0.04
Ketones	C	2-hexanone	1.35 ± 0.05	0
	G	cyclohexanone	4.60 ± 0.00	4.74 ± 0.02
	N	methylacetophenone	0.39 ± 0.04	0

[a]The value of integrated peak areas X (in arbitrary units) was transformed to $X' = \log (X + 1)$.
[b]Compound not present in the sample even in trace quantities.

experiments on these compounds should be conducted to evaluate their role, if any, in white-tailed deer communication.

Three of the 14 characteristic compounds occurred at similar levels in both estrous and mid-cycle mucus samples. These included two alcohols (2-butanol, cyclohexanol) and a ketone (cyclohexanone). Interestingly, cyclohexanone was present in both estrous and mid-cycle samples and at far higher concentration (40–92%, $P < 0.02$), than other compounds (Table 1).

The remaining 30 vaginal mucus volatiles also were found in urine samples (Table 2 and Table 5 below). Concentration of these compounds did not vary significantly between estrus and mid-cycle urines ($P > 0.05$; Table 2 shows the mean urinary value for each compound). In the mucus, 15 of these compounds varied depending on whether the mucus was discharged by estrous or mid-cycle females and tended to be lower than in the urine (Table 2). These volatiles included alcohol, aldehyde, alkane, and ketone structural classes, and all were ovarian hormone-dependent. Concentrations of 11 compounds in the estrous mucus were lower ($P < 0.02$) when compared to mid-cycle mucus, but concentration of only four mid-cycle mucus compounds was lower ($P < 0.02$) when compared to estrous mucus (Table 2). Only 1-octen-3-ol (peak 51) from

TABLE 2. DIFFERENCES IN CONCENTRATION OF VOLATILES COMMON FOR MUCUS AND URINE

Class of compounds and peak number	Structure	Integrated peak areas (mean ± SEM)[a]		
			Mucus	
		Urine[b]	Estrus	Mid-cycle
Alcohols				
44[c]	3-methyl-1,3-butanediol	1.99 ± 0.01b	0.86 ± 0.13c	2.83 ± 0.02a
51	1-octen-3-ol	1.67 ± 0.03c	2.15 ± 0.19a	1.87 ± 0.01b
65	alpha-terpineol	2.44 ± 0.01a	1.39 ± 0.01b	2.13 ± 0.01a
Aldehydes				
15[c]	valeraldehyde	2.20 ± 0.02a	1.04 ± 0.02c	1.39 ± 0.01b
21	hexanal	2.70 ± 0.00a	2.42 ± 0.02a	1.74 ± 0.05b
30	heptanal	2.72 ± 0.01a	1.99 ± 0.00b	2.69 ± 0.00a
41	octanal	3.67 ± 0.01a	2.41 ± 0.04c	2.68 ± 0.02b
49	nonanal	4.62 ± 0.04a	3.01 ± 0.01c	3.50 ± 0.02b
Alkanes				
9[c]	octane	2.25 ± 0.00a	1.09 ± 0.01c	1.41 ± 0.04b
10	1,2,4-trimethyl-cyclopentane	2.80 ± 0.04a	1.75 ± 0.01c	2.00 ± 0.01b
45	dodecane	2.26 ± 0.02a	2.12 ± 0.01a	1.02 ± 0.01b
Ketones				
29	2-heptanone	1.99 ± 0.02a	0.57 ± 0.01c	0.89 ± 0.03b
42[c]	7-methyl-4-octanone	1.90 ± 0.01a	1.39 ± 0.03b	1.00 ± 0.01c
43	6-methyl-5-hepten-2-one	2.41 ± 0.01a	1.25 ± 0.01b	1.45 ± 0.02b
48[c]	2-nonanone	1.65 ± 0.01a	0.45 ± 0.00c	0.72 ± 0.01b

[a] The value of integrated peak areas X (in arbitrary units) was transformed to $X' = \log(X + 1)$. Means in the same row not followed by the same letter are significantly different [$F(2,6)$; $P < 0.02$)].

[b] Concentration of the urinary compounds listed in this column did not vary significantly between estrus and mid-cycle samples ($P > 0.05$); thus for comparison of urine with mucus samples, the mean urinary value was used for each compound.

[c] These volatiles were not present in the urine of ovariectomized females even in trace quantities.

estrous mucus and 3-methyl-1,3-butanediol (peak 44) from mid-cycle mucus were significantly greater (45% and 43%, respectively; $P < 0.02$) in concentration than urinary level (Table 2).

In addition to the 15 volatiles previously described, common for mucus and urine (Table 2), we found 33 more compounds that were consistently present only in urine (Tables 3 and 4). The GC-MS recording shown in Figure 1b is typical of the volatile profiles obtained from estrous female urine. Of the total 63 investigated urinary compounds (Tables 2–5), 46 occurred in both reproductive stages; 13 were present in estrous urine only and four in mid-cycle samples

TABLE 3. VOLATILE COMPOUNDS[a] CHARACTERISTIC OF URINE

Class of compounds	Peak number	Structure	Integrated peak areas (mean ± SEM)[b] Estrus	Mid-cycle
Alcohol	50	2-octanol	3.92 ± 0.00	0[c]
Aldehydes	4	acrolein	0.48 ± 0.00	0
	55	2-nonenal	4.49 ± 0.00	0
	69	dodecanal	2.44 ± 0.00	0
Alkanes	1	hexane	2.16 ± 0.01	0
	2	2-methylhexane	1.77 ± 0.01	0
	27	identity uncertain	1.21 ± 0.01	0
Alkenes	11	4-octene	0.91 ± 0.01	0
	12	MW 112	1.26 ± 0.01	0
	35	limonene	1.59 ± 0.01	0
	47	identity uncertain	1.95 ± 0.01	0
Furans	36	2-pentylfuran	1.65 ± 0.01	0
	66	2-(4-hydroxybutyryl) furan	0	1.75 ± 0.01
Ketones	56	acetophenone	0	0.75 ± 0.01
	60	2-cyclohexenone	1.33 ± 0.01	0
Pyran	3	2-methyl-2-3-dihydropyran	0	2.28 ± 0.01
Unidentified	58	identity uncertain	0	2.43 ± 0.01

[a] Urinary volatiles listed in this table were not present in the urine of ovariectomized females even in trace quantities.
[b] The value of integrated peak areas X (in arbitrary units) was transformed to $X' = \log (X + 1)$.
[c] Compound not present in the sample even in trace quantities.

only (Table 3). Volatiles occurring exclusively in estrus urine represented a variety of chemical groups including alcohols, aldehydes, alkanes, alkenes, furans, and ketones. Those characteristic of mid-cycle urine belonged to the furan, ketone, and pyran groups (Table 3).

Many of the urinary compounds appeared to be hormone-dependent. Thirty-eight of 63 compounds (60%) were absent, not found even in trace quantities, from urine of ovariectomized females (Tables 2–4). The only compounds with significantly greater concentration in ovariectomized females were an alcohol [1-octen-3-ol (peak 51)] and an alkane [1,2,4,-trimethylcyclopentane (peak 10)]. Those compounds are common for both mucus and urine samples (Table 2).

Among the volatiles identified in mucus (total 44) and urine (total 63), several compounds were present in all investigated samples. Concentrations of these volatiles were similar in all samples (i.e., 1.24–2.73 log of integrated peak areas in arbitrary units), were relatively high, and were not dependent on ovarian hormones or animals' reproductive stage (Table 5).

TABLE 4. OVARY-DEPENDENT URINARY VOLATILES[a]

Class of compounds	Peak number	Structure
Alcohols	31	1-penten-3-ol
	46	1-hexanol
	52	2-octen-1-ol
	62	a monoterpene alcohol
Aldehydes	6	methacrolein
	7	butyraldehyde
	57	methylbenzaldehyde
	63	undecanal
	64	2,4-nonadienal
Alkanes	23	identity uncertain
	59	tetradecane
Alkene	18	identity uncertain
Ketones	32	6-methyl-2-heptanone
	39	2-octanone
Phenol	73	p-ethylphenol
Unidentified	37	identity uncertain

[a]Urinary volatiles listed in this table were not present in the urine of ovariectomized females even in trace quantities.

TABLE 5. VOLATILE COMPOUNDS COMMON TO BOTH VAGINAL MUCUS AND URINE SAMPLES[a]

Class of compounds	Peak number	Structure
Alkenes	67	pentadecane
	70	hexadecane
	72	heptadecane
Amine	68	*N*-ethylaniline
Ester	71	propylene dibutyrate
Ketones	13	2-pentanone
	14	3-pentanone
	17	2,3-pentanedione
	20	2-hexanone
	24	cyclopentanone
	25	4-heptanone
	28	3-methylcyclopentanone
	34	2-methyl-4-heptanone
	38	3-ethylcyclopentanone
	61	phenylacetone

[a]They demonstrated relatively constant concentration in samples of all investigated females.

Limited research has been conducted on the urinary volatiles of cervids. Bakke and Figenschou (1990) reported 70 volatile substances in adult red deer (*Cervus elaphus elaphus*) from gas chromatography of a methylated acidic fraction of urine, a technique different from the methods used in the present study. Their substances were mainly carboxylic acids and their derivatives, in addition to some aromatic compounds. They did not observe any systematic variation in volatile excretion during different seasons. In black-tailed deer (*O. hemionus hemionus*), Muller-Schwarze et al. (1978) presented evidence that a urinary γ-lactone, (Z)-6-dodecen-4-olide, is involved in social communication. Thus, the results presented here provide additional chemical background information for the future testing of animal behavior.

CONCLUSIONS

Certain compounds present in vaginal mucus are not paralleled in the urine of white-tailed deer. In addition, several compounds found in estrous mucus were not found in mid-cycle samples. Other volatiles were found in both urine and vaginal mucus, but their concentration tended to be lower in mucus samples. These overlap compounds found in both mucus and urine (for both phases of the female reproductive cycle) suggest that some compounds in mucus sample may have originated from urine contamination.

Our study documents for the first time that: (1) the composition of volatiles in white-tailed female deer changes according to the stage of the reproductive cycle; (2) estrous mucus and urine have more characteristic compounds (9 and 13, respectively) then mid-cycle mucus and urine samples (2 and 4, respectively), and these compounds may be used to identify a particular stage of the deer's reproductive cycle; and (3) numerous urinary compounds exhibit strong dependence on ovarian hormones.

Further biological and behavior research is needed to determine which chemical compounds identified in this study (if any) are important in communicating sexual/reproductive status.

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