CHEMICAL ANALYSIS OF PREOVULATORY FEMALE AFRICAN ELEPHANT URINE: A SEARCH FOR PUTATIVE PHEROMONES

Thomas E. Goodwin, L. E. L. Rasmussen, Bruce A. Schulte, Patrick A. Brown, Ben L. Davis, Whitney M. Dill, Nichole C. Dowdy, Adam R. Hicks, Richard G. Morshedi, Daniel Mwanza, and Helen Loizi *

1. INTRODUCTION

Many similarities exist between the lifestyles and behaviors of *Loxodonta africana* and *Elephas maximus*, two of the three extant species of elephants (Sukumar, 2003; Poole, 1987, 1989a,b; Rasmussen and Krishnamurthy, 2000; Rasmussen and Schulte, 1998). While the roles of olfaction and the chemical senses in Asian elephant society have been extensively investigated (Rasmussen and Greenwood, 2003; Rasmussen et al., 1997, 2002), similar investigations in the African species are limited to recent studies of chemical signals among males (Rasmussen and Wittemyer, 2002).

Male elephants face reproductive challenges not only of locating females, as the sexes live somewhat separated, but also of detecting the most fertile period of females, i.e. as they approach ovulation during the 13-17 week estrous cycle. The Asian species has been shown to utilize a urinary pheromone (Rasmussen et al., 1996). Commencing fairly early in the follicular phase, low concentrations of an acetate pheromone attract males (Rasmussen, 2001). The available urinary concentration of this ligand elevates gradually as ovulation approaches. Males apparently can measure quantitatively the pheromone concentration and thus the female's proximity to ovulation. This is evidenced by high frequencies of flehmen responses and premating behaviors. Based on selected field observations and unpublished data on captive elephants, our group recently began a rigorously designed multiple-elephants and multiple-sites study to establish whether male

* Thomas E. Goodwin, Patrick A. Brown, Ben. L. Davis, Whitney M. Dill, Nichole C. Dowdy, Adam R. Hicks, Richard G. Morshedi, and Daniel Mwanza, Hendrix College, Conway, AR, 72032. L. E. L. Rasmussen, Oregon Health and Sciences University, Beaverton, OR, 97006. Bruce A. Schulte and Helen Loizi, Georgia Southern University, Statesboro, GA, 30460.

African elephants are chemosensorily attracted toward females; in particular whether they are attracted by urinary chemical signals at specific periovulatory periods.

The African species has a similar three-to-four month estrous cycle, characterized by an ovulatory elevation of luteinizing hormone (LH2) coinciding with the initial rise, slight decrease, then sustained elevation of serum progestins, and an anovulatory luteinizing hormone peak (LH1) about three weeks earlier (Brown, 2000). We have hypothesized that related to these two hormonal elevations, urinary chemical signals are released by female African elephants that attract males and signal impending ovulation.

Our research strategy began with the acquisition of estrous-defined urine samples from captive female African elephants. After such collections, chemical analyses were initiated to search for a putative pheromone employing four differently emphasized search strategies: (1) Identify compounds known to be pheromones in other species; (2) Identify compounds seen exclusively or largely around LH1 or LH2 elevations; (3) Identify compounds seen exclusively or largely in protease- or acid-treated samples; (4) Identify compounds that are predominant in urine samples demonstrated to be bioactive. Because our behavioral study is long-term and of a design and sample size appropriate to tease out robust chemosensory responses, we were not able to use the fourth strategy in the current report. This paper presents some of our initial chemical findings as examples typical of the data being gathered.

2. MATERIAL AND METHODS

2.1. Urine Collections

Our defined requirements for urine acquisition included collections from regularly cycling females whose serum progesterone (P4) was monitored weekly and serum LH monitored daily during the three-week period immediately preceding ovulation. Collection days were selected after reviewing the chronology of serum LH and P4 concentrations during previous estrous cycles. At least one cycle and sometimes multiple cycle histories were required to anticipate the timing of the two LH peaks. On each day of collection, concurrent serum samples were obtained to confirm subsequently the hormonal status of the female. Our schedule for each female included collection on the following days: two luteal phase days; day of LH1 and day before and after; day of LH2 and day before and after; a day midway between LH1 and LH2. On each collection day multiple mid-stream urine aliquots were collected in clean glass containers and immediately frozen on dry ice or placed in a -80 °C freezer and stored at –80 °C until analysis. Institutions participating in these collections are listed in the Acknowledgments section.

2.2. Chemical Analyses: Sampling Methods and Analysis Procedures

We utilized two sampling methods combined with gas chromatography-mass spectrometry (GC-MS) to identify urinary compounds. Our adsorption and absorption sampling and capture techniques allowed us to encompass a substantial molecular weight (MW) range. We spanned in a somewhat selective manner from small compounds such as acetaldehyde (MW = 44) and trimethylamine (MW = 59) up to large ones such as steroids (MW over 300). For the more volatile compounds, evacuated canister capture

followed by cryogenic trapping (ECC/CT) prior to GC separation was employed. Less volatile compounds were trapped by solid phase microextraction (SPME) by two slightly different procedures, an automated one conducted at the laboratory of Dr. Goodwin and his students, and a manual one at the laboratory of Dr. Rasmussen. These procedures are described in more detail below.

2.2.1. Sampling: ECC/CT

One hundred mL of urine was placed in a 500-ml clean glass jar fitted with a special lid that contained two Swagelok fittings. One fitting was connected to the jar via ultra-clean Nupro SS-4H4 bellow-stem valves to a special stainless steel receiving bottle (0.85 or 6 L) evacuated to ~30 inches Hg vacuum. Similar valves connected the other fitting to a source bottle of pure air pressured to 40 psig. Prior to starting the experiment, pure air was flushed into the system. Next, the jar samples were heated to 37 °C (mimicking elephant body temperature) and allowed to equilibrate for 30 min, thus allowing the development of headspace volatility. Subsequently, at 30-min intervals for 2.5 h (and on occasion for longer inter-sample intervals and up to a total time of 24 h), the stainless steel evacuated receiving bottle was briefly opened to allow the entry of compounds developed in the headspace. At the end of the collection time, the receiving canisters were pressurized with helium to 30 psig to ensure long-term storage at room temperature and to facilitate GC/MS analyses. Further details are provided in Perrin et al. (1996) and Rasmussen and Perrin (1999).

The sample introduction system for subsequent GC/MS analyses of urine headspace volatiles contained within these pressurized stainless steel canisters involved the initial release of the volatiles from the canister and their adsorption onto an in-line Tenax trap. In turn, desorption from the Tenax, employing a six-port valve in line with a U-tube cryogenic trap (0.125 in OD x 9 in) containing 60/80 mesh glass beads, was followed by cryogenic focusing on this loop. Compounds were then released from the loop by heat, and separated by gas chromatography.

2.2.2. Analysis: GC/MS Following ECC/CT

The ECC/CT samples were analyzed on a Hewlett-Packard 5890A GC and a Hewlett-Packard 5970B MS. The GC used a DB-1, 0.25-mm ID x 60 m x 1.0 μm film thickness, polymethyl silicone-coated capillary column (J & W Scientific). The gas chromatograph oven was temperature programmed from –60 to 200 °C at 4°C/min. The mass spectrometer was programmed for a mass scan of 33–300, which allowed for identification of compounds from C3 through C14. Compounds were identified using an NBS 75 K Hewlett-Packard Mass Spectrometer ChemStation library search and were manually rechecked with the NIST/EPA/NIH Mass Spectral Data Base Version 4.01 and the Wiley Library Version 6-275. In addition to assigning compound identity based on mass (molecular) ion and dominant ion patterns, a number of internal standards of authentic compounds were employed for compounds of interest. Although 75% match was the minimum criterion, most compound matches were greater than 90%. ECC/CT-GC/MS was utilized primarily to identify the dominant volatile compounds and although quantitation was not conducted for this study, this method, with greater numbers of duplicate samples, will be used in the future for quantitation of lower molecular weight volatiles.

2.2.3. Sampling: Manual Solid Phase Microextraction (SPME)

Aliquots (500 µl) of urine were sampled by manual SPME prior to GC/MS. The vials used for SPME were steam-cleaned by hot distilled water, rinsed three times with triple-distilled water, and air-dried prior to the addition of urine. Either a reverse-direction insert top, conditioned for several days in a GC oven at 250 °C, or an aluminum foil cover through which the fiber and its holder were inserted was used. Based on the results from Asian female urine (Rasmussen, 2001), 100-µm polydimethylsiloxane (PDMS) SPME fibers were employed. No fibers were immersed; rather, they were exposed in the headspace above the liquid sample, which was gently stirred by a tiny magnetic stirring bar. For each sample, the adsorption of volatile compounds on the fiber was conducted first at native pH and at ambient temperature (25 °C), and then heated to 37 °C. For selected duplicate samples, 1 mg/ml of non-specific bacterial protease (Sigma cat. no. P-5147) was added (Poon et al., 1999; Yamazaki et al., 1999). For some of these samples, the pH was adjusted to 4.0, the pH demonstrated to result in release of ligands from urinary albumin (Lazar et al., 2002). Selected samples were also reduced further to pH 1.0.

Adsorption times were 1 h or more for each condition, at which time the fiber was retracted into the protective needle. Immediately, the SPME needle was inserted into the injector port of the GC outfitted with a special glass liner insert, and the fiber was exposed for 10 min to allow desorption at 250 °C, the temperature of the injector port. Thus, compounds adsorbed on the PDMS fiber were desorbed and focused onto the beginning of the 40 °C GC column. Because of the tendency of some analytes to adhere to glass or plastic surfaces (Prestwich, 1987), three blank samples were run prior to the analyses of samples, and fibers were reconditioned twice between analyses with a blank analysis conducted after the second conditioning. The glass liner was also cleaned and replaced weekly.

2.2.4. Analysis: GC/MS Following Manual SPME

GC/MS analyses for the manual SPME experiments were conducted using a Hewlett-Packard 6890A GC and a Hewlett-Packard 5973 mass selective detector. The GC column was identical to that used for ECC/CT. The GC oven was temperature programmed from a 4 min hold at 40 °C to 200 °C at 6 °C/min, then ramped at 2 °C/min to a final temperature of 235 °C.

The mass spectrometer was programmed at 0.83 scans/sec for a mass scan of 33–550, which allowed for identification of compounds from C3 through C18. Most compounds were identified using an NBS 75 K Hewlett-Packard Mass Spectrometer ChemStation library search and were manually rechecked with the NIST/EPA/NIH Mass Spectral Data Base Version 4.01 and the Wiley Library Version 6-275. Mass (molecular) ion and dominant ion patterns by mass spectrometry were of primary importance in assigning identity. The criterion of matching was at least 75% and most matches were in the 90% range. Selected internal standards also were analyzed. SPME followed by GC/MS was used for identification of higher molecular weight compounds, but there was no attempt at quantitation.

2.2.5. Sampling: Automated SPME

Aliquots of urine were sampled by automated SPME prior to GC/MS. Sampling was carried out with a Gerstel Multipurpose Sampler (MPS2). New 20 mL vials (Gerstel part no. GC 93640-06) were rinsed three times with reagent grade acetone, rinsed three times with deionized water, and oven-dried (110 °C) prior to the addition of urine (1 mL). Normally the urine was saturated with solid NaCl, and then the vial was crimp-sealed using magnetic crimp caps (Gerstel part no. 093640-008-00). In some instances, urine samples were treated with 1 mg/mL of non-specific bacterial protease (Sigma, cat. no. P-5147) (Poon et al., 1999; Yamazaki et al., 1999). For other samples, 1M HCl was used to adjust the pH to 3 or 4 (down from the natural pH of 7-8), the pH demonstrated to result in release of ligands from urinary albumin in Asian elephants (Lazar et al., 2002).

Based on the results from Asian female urine (Rasmussen, 2001), 100-μm polydimethylsiloxane (PDMS) SPME fibers were used (23 gauge needle for autoholder and Merlin Microseal™ septum, Supelco part no. 57341-U; 1.0 mm ID GC inlet liner, Resek part no. 20973). The SPME fiber was not immersed, but rather exposed for 30 min in the headspace above the liquid sample that was heated at 37 °C and agitated at 250 rpm. The MPS2 then retracted the SPME fiber into the protective needle and transferred it to the GC inlet where it was exposed and desorbed at 250 °C for 10 min under splitless conditions, then for an additional 10 min under split conditions to clean the fiber. In trial runs and test cases, no cross-contamination (ghosting) from run to run was detected when using this protocol. Normally, five samples were analyzed prior to cleaning the fiber and column with a blank run. The glass inlet liner was cleaned as needed.

2.2.6. Analyses: GC/MS Following Automated SPME

GC/MS analyses were conducted using an Agilent 6890N GC and 5973N mass selective detector. The capillary GC column was an SPB-1 (bonded; poly(dimethylsiloxane)), 60 m x 0.32 mm ID, 1 μm film thickness (Supelco cat. no. 24047). The GC oven was temperature programmed from a 20-min hold at 45 °C (20 min is the total SPME fiber desorption time) to 110 °C at 6 °C/min, held at that temperature for 10 min, then ramped at 2 °C/min to a final temperature of 210 °C where it was held for 45 min. The mass spectrometer was programmed at 2.86 scans/sec for a mass scan of 35–550. Most compounds were identified using the NIST02 mass spectral library. Additional searching was carried out with the Wiley Library Version 6-275. Mass (molecular ion) and dominant fragmentation ions were of primary importance in assigning identity. Although 75% match was the minimum criterion, most compound matches were greater than 90%.

3. RESULTS AND DISCUSSION

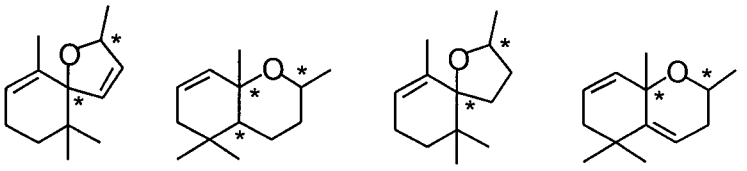
Two new techniques for the analysis, i.e. identification and quantification, of chemical compounds in animal secretions and excretions offer decisive advancements in investigations of mammals whose social actions and reproduction are well-known behaviorally. The largest terrestrial herbivores, elephants, with an enormous impact on other nearby animals and on their habitat, have been the focus of extensive behavioral studies involving both wild and captive populations. In both the Asian and African

savannah species, males are known to be attracted to estrous females (Eisenberg et al., 1971; Moss, 1983). In the Asian species, the multiplicity of flehmen responses by males to preovulatory females was well-documented for eight years and correlated with the establishment through serum hormone measurements of the approximately three to four month cyclicity of the estrous cycle (Hess et al., 1983). Recently a similar female estrous cyclicity has been demonstrated for African female elephants (Brown, 2000). For the Asian species, the extensive record allowed the initiation in 1980 of an attempt to identify the active chemical signal(s) that elicited the flehmen responses by males (Rasmussen et al., 1982). The resultant investigation involving bioassay-guided fractionations was long and tedious, sustained, however, by the robust bioresponses by all male elephants tested (Rasmussen et al., 1986).

Such bioassays of urinary components separated by organic chemical extraction, flash chromatography, and high pressure liquid chromatography resulted in identification of a female-to-male preovulatory pheromone of the Asian elephant (Rasmussen et al., 1982, 1986, 1996). Many compounds in the urine from the luteal and follicular phases of captive females were identified by GC-MS and assayed. Elevated, or prominent, in preovulatory urine were numerous aldehydes, ketones, alcohols, and phenols; 4-methylphenol and 4-ethylphenol especially were a high proportion of these organic extracts (Rasmussen et al., 1997). However, many active fractions were dominated by an unknown entity with a mass of 166. It was suspected that this mass was a fragment of a larger molecule, perhaps an acetate. Final identification, in 1996, was confirmed through the use of a new technique, solid phase microextraction (SPME) (Arthur and Pawliszyn, 1990) to identify the whole molecule, (*Z*)-7-dodecenyl acetate (*Z*7-12:Ac). Only *Z*7-12:Ac exhibited two features: (1) a 1000-fold increase in concentration occurring primarily between two preovulatory luteinizing hormone elevations, and (2) robust bioactivity (Rasmussen et al., 1997; Rasmussen, 2001). With the new techniques available and our four-pronged research approach, we are now able to demonstrate characteristic compounds in female African elephant urine and to suggest some potentially fruitful research directions.

Tables 1 and 2 provide one example of GC-MS results from analysis of the same urine sample by ECC/CT and SPME, respectively. Clearly, ECC/CT facilitates the observation of the more volatile organic compounds. Tables 3 and 4 illustrate typical results from automated SPME/GC-MS analysis of a urine sample at physiological pH 8 versus pH 3. It is noteworthy that the compounds listed in Table 4 (pH 3) at retention times of 64.49, 65.40, 65.69, 66.24, 66.49, 67.24, and 67.89 minutes constitute a total of 62.48 area percent of the total products observed, while none of these compounds appear at all in the pH 8 sample. The compound at 64.49 minutes is a known synthetic spirocycle (**1**) that has not been observed previously as a natural product (Ehrenfreund et al., 1974; Renold et al., 1975; Schulte-Elte, et al., 1978). This is not the first time that we have observed unique natural products in African elephant secretions and excretions (Goodwin et al., 1999, 2002).

The compounds at retention times 65.40, 65.69, and 67.89 minutes (Table 4) represent three of the four possible diastereomers of the natural products commonly known as dihydroedulans (**2**). The compounds at retention times 66.24 and 67.24 minutes are the diastereomeric theaspirane (**3**) natural products. Finally, the compound at a retention time of 66.49 minutes represents one of the two diastereomers of the natural product edulan (**4**).



(1) (2) (3) (4)

(*chirality centers)

Table 1. Compounds identified (>74% match quality) in LH1 urine (3/11/03) of Alice[a] by ECC/CT and GC-MS (physiological pH = 8, 37 °C, NaCl added).

Compound	Ret. Time (min.)	Compound	Ret. Time (min.)
Acetaldehyde	12.25	3-Penten-2-one	35.74
Ethanol	18.97	Dimethyl disulfide	36.11
Acetone	20.13	Pyrrole	36.38
2-Propanol	21.38	3-Methylthiophene	37.85
Dimethyl sulfide	22.25	3-Hexanone	38.21
2-Methylpropanal	24.57	2-Hexanone	38.36
2-Butenal	25.31	Hexanal	38.89
3-Buten-2-one	26.25	Isopropyl isothiocyanate	40.45
Butanal	26.64	3-Methylcyclopentanone	41.11
2-Butanone	26.97	4-Heptanone	42.86
2-Methyl-3-buten-2-ol	28.51	2-Heptanone	43.75
2-Methyl-1-propanol	29.67	Heptanal	44.18
3-Methylbutanal	30.64	3-Ethylcyclopentanone	46.74
3-Methyl-2-butanone	31.00	2,3-Octanedione	47.99
2-Methylbutanal	31.28	Acetophenone	51.52
3-Methyl-3-buten-2-one	31.81	2-Octen-4-one	54.39
1-Penten-3-one	32.43	2-Cyclohexen-1-one, 2-methyl-5-(1-methylethenyl)-	59.36
2-Pentanone	32.57	2-Cyclohexen-1-one, 3-methyl-6-(1-methylethyl)-	59.85
Pentanal	33.10	2-Buten-1-one, 1-(2,6,6-trimethyl-1,3-cyclohexadien-1-yl)-, (E)-	64.79
4-Methyl-2-pentanone	35.73	3-Buten-1-one,4-(2,6,6-trimethyl-1-cyclohexen-1-yl)	65.93

[a]Female African elephant (age 33 yrs. at time of sampling), Wildlife Safari Park, Winston, OR.

Table 2. Compounds identified (>74% match quality) in LH1 urine (3/11/03) of Alice[a] by automated SPME and GC-MS (physiological pH = 8, 37 °C, NaCl added).

Compound	Ret. Time (min.)
Isopropyl isothiocyanate	29.02
2,3-Octanedione	38.70
Benzene, 1-methyl-4-(1-methylethyl)-	43.31
2-Cyclohexen-1-one, 3-methyl-	43.96
Phenol, 4-methyl-	45.86
Phenol, 2-ethyl-4,5-dimethyl-	50.10
5-Isopropyl-3,3-dimethyl-2-methylene-2,3-dihydrofuran	52.91
Phenol, 3-ethyl-	53.49
4,7-Dimethylbenzofuran	58.73
Cyclohexanone, 5-methyl-2-(1-methylethylidene)-	60.15
2-Cyclohexen-1-one, 3-methyl-6-(1-methylethyl)-	61.14
2-Cyclohexen-1-one, 3-methyl-6-(1-methylethylidene)-	67.36
2-Buten-1-one, 1-(2,6,6-trimethyl-1,3-cyclohexadien-1-yl)-, (E)-	70.99
5,9-Undecadien-2-one, 6,10-dimethyl-, (E)-	75.20

[a]Female African elephant (age 33 yrs. at time of sampling), Wildlife Safari Park, Winston, OR.

The edulans and dihydroedulans were first identified in passionfruit (Whitfield and Stanley, 1977; Prestwich et al., 1976). Subsequently, edulans were seen in human urine (Mills and Walker, 2001), while dihydroedulans have also been found in male scent organs of African butterflies (Schulz et al., 1993). The theaspiranes have been identified in green and black tea, as well as in a number of fruits and berries (Schmidt et al., 1992). Later, a theaspirane was observed by SPME/GC-MS in urine from a female Asian elephant (Rasmussen, 2001). Recently, a dihydroedulan and a theaspirane were reported from giant panda urine (Dehnhard et al., 2003).

At this point neither the origin of compounds **1-4** in elephant urine, nor their role, if any, in chemical signaling among elephants is clear. It is noteworthy that in a previous study of the isolation of volatiles from quince fruit, high vacuum distillation/extraction followed by GC-MS yielded different results if one started with homogenized fruit at its natural pH (3.7), versus homogenate at pH 7 to which an enzyme inhibitor had been added. Specifically, the pH 3.7 sample evidenced large amounts of the theaspiranes, but the pH 7 sample showed only trace amounts (Winterhalter et al., 1987).

Additionally, it has been demonstrated that under acidic conditions, certain monocyclic diols can be converted to the theaspiranes (**3**), or to the dihydroedulans (**2**), and that the latter (**2**) may be isomerized to the former (**3**) on strong acid treatment (Schulte-Elte et al., 1978; Winterhalter et al., 1987; Schmidt et al., 1995; Young et al., 2000). Thus it is possible that in African elephant urine at pH 3-4, we are observing products (**1-4**) that are formed from as yet unidentified precursors in the native pH 8 urine. If so, it is likely that the precursors are degradation products of carotenoids, as has been suggested for similar bisnorsesquiterpenes (Francke et al., 1989; Kaiser and Lamparsky, 1979).

Table 3. Compounds identified (>74% match quality) in LH2 urine (9/11/01) of Timba[a] by automated SPME and GC-MS (physiological pH = 8, 37 °C, NaCl added).

Compound	Ret. Time (min.)
Benzene, 1,3-dimethyl-	32.51
p-Xylene	32.79
Benzaldehyde	36.86
2,3-Octanedione	38.82
Benzyl chloride	41.38
Benzene, 1-methyl-4-(1-methylethyl)-	43.43
Phenol, 4-methyl-	45.97
Benzene, 1-methyl-4-(1-methylethenyl)-	48.60
3-Methyl-4-isopropylphenol	50.22
1,3,8-p-Menthatriene	50.79
Acetophenone, 4'-hydroxy-	53.47
Benzene, 1-methyl-3-(1-methylethyl)-	54.29
3-Methyl-2,3-dihydro-benzofuran	55.39
Bicyclo[3.2.0]hept-3-en-2-one	64.91
2-Buten-1-one, 1-(2,6,6-trimethyl-1,3-cyclohexadien-1-yl)-, (E)-	71.10
2H-1-Benzopyran-2-one	73.07
3-Buten-1-one, 4-[2,6,6-trimethyl-1(or 2)-cyclohexen-1-yl]-	73.26
1,6,6-Trimethyl-7-(3-oxobut-1-enyl)-3,8-dioxatricyclo[5.1.0.0(2,4)]octan-5-one	74.67

[a]Female African elephant (age 24 yrs. at time of sampling), Seneca Park Zoo, Rochester, NY.

In two pheromonal systems in the Asian elephant, proteins play transport and sequestering roles prior to pheromonal interactions with sensory receptors. Frontalin, a chemical signal of musth in older males, is linked to elephant albumin in the temporal gland secretion at appropriate pHs (Rasmussen et al., 2003). Likewise, Z-7-dodecenyl acetate (Z7-12:Ac), the preovulatory urinary pheromone, is bound to urinary albumin, maximally at alkaline pH. The latter pheromone has been more thoroughly investigated, revealing an interesting synchrony of events in the urine of the female prior to ovulation (Rasmussen, 2001). Not only does the concentration of Z7-12:Ac rise during this periovulatory period between transitory elevations of serum luteinizing hormone, but also urinary pH becomes more alkaline and protein content/creatinine levels elevate. Surprisingly, there are few low molecular mass, lipocalin-like proteins in the female urine (Lazar, 2001). Instead, a 66-kDa urinary protein is preferentially bound to the pheromone as studied by SDS and native gel electrophoresis (Lazar et al., 2002). Our preliminary studies suggest that female African elephant urine may undergo similar changes. We are modeling the demonstrated binding of Asian elephant pheromones to urinary albumin as an exploratory tool in our search for chemical signals operational from female African elephants toward conspecific males.

4. ACKNOWLEDGMENTS

Elephant urine samples were provided by the following organizations: Cameron Park Zoo, Indianapolis Zoo, Louisville Zoo, Nashville Zoo (R. and C. Pankow), Riddle's Elephant Sanctuary, Sedgwick County Zoo, Seneca Park Zoo, Six Flags Marine World,

Table 4. Compounds identified (>74% match quality) in LH2 urine (9/11/01) of Timba[a] by automated SPME and GC-MS (pH = 3, 37 °C).

Compound	Ret. Time (min.)
4-Heptanone	31.82
Oxepine, 2,7-dimethyl	35.89
1-Hexen-3-yne, 2,5,5-trimethyl-	40.56
Cyclohexene, 3-methyl-6-(1-methylethylidene)-	41.34
Benzene, 1-methyl-2-(1-methylethyl)-	43.31
1,4-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-	46.43
Benzene, 1-methyl-4-(1-methylethenyl)-	48.49
Bicyclo[4.1.0]hept-2-ene, 3,7,7-trimethyl-	49.01
1,3,8-p-Menthatriene	50.66
Benzofuran, 2,3-dihydro-2-methyl-	52.80
Benzene, 4-ethyl-1,2-dimethyl-	54.16
Benzenemethanol, .alpha.,.alpha., 4-trimethyl-	55.62
Benzene, 2-(2-butenyl)-1,3,5-trimethyl-	58.12
Naphthalene, 1,2,3,4-tetrahydro-1,1,6-trimethyl-	59.46
1-Oxaspiro[4.5]deca-3,6-diene, 2,6,10,10-tetramethyl-	64.49
2-Oxabicyclo[4.4.0]dec-9-ene, 1,3,7,7-tetramethyl-	65.40
2H-1-Benzopyran, 3,4,4a,5,6,8a-hexahydro-2,5,5,8a-tetramethyl-(2.alpha.,4a.alpha.,8a.alpha.)-	65.69
2,6,10,10-Tetramethyl-1-oxa-spiro[4.5]dec-6-ene	66.24
2H-1-Benzopyran, 3,5,6,8a-tetrahydro-2,5,5,8a-tetramethyl-, trans	66.49
2,6,10,10-Tetramethyl-1-oxa-spiro[4.5]dec-6-ene	67.24
2H-1-Benzopyran, 3,4,4a,5,6,8a-hexahydro-2,5,5,8a-tetramethyl-(2.alpha.,4a.alpha.,8a.alpha.)-	67.89
1H-Indene, 2,3-dihydro-1,1,5,6-tetramethyl-	69.22
Naphthalene, 1,2-dihydro-1,1,6-trimethyl-	69.44
Benzene, 1,1'-(1,1,2,2-tetramethyl-1,2-ethanediyl)bis[4-methyl-	69.88
Benzene, 1,4-dimethyl-2,5-bis(1-methylethyl)-	70.03
Naphthalene, 1,2-dihydro-1,4,6-trimethyl-	71.84
Benzene, 2-(1,3-butadienyl)-1,3,5-trimethyl-	72.08
Naphthalene, 2,6-dimethyl-	72.84

[a]Female African elephant (age 24 yrs. at time of sampling), Seneca Park Zoo, Rochester, NY.

and Wildlife Safari Park. We thank the National Science Foundation for financial support. We are grateful to Scott and Heidi Riddle for their advice and for facilitating observations at Riddle's Elephant Sanctuary. We appreciate the gift of several chemical samples from Augustus Oils Limited. Mary Wiese provided valuable assistance in the preparation of this manuscript.

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