manatee. For example, interhemispheric asymmetry of slow waves occupied approximately a quarter of the total slow wave sleep time in a 24-h period. In contrast to dolphins and Otariidae seals, in which, as we suggested previously[1-3], interhemispheric asymmetry of ECoG slow waves is due to retained locomotory activity during sleep, no movements were associated with the slow wave interhemispheric asymmetry in the Amazonian manatee. The functional significance of this ECoG pattern therefore remains unknown.

The first study of sleep in a representative of the order of Sirenia showed that some features of its sleep were similar to those found before in other aquatic mammals. Thus the aquatic mode of life resulted in considerable changes of sleep structure in Cetacea, Sirenia, and Pinnipedia as compared to terrestrial mammals.

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Pheromone binding proteins of the mouse, *Mus musculus*

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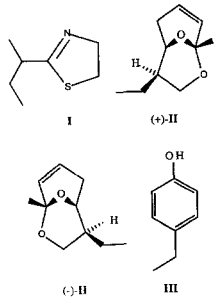
Abstract. Proteins of the Major Urinary Complex of the adult male mouse (*Mus musculus*) selectively bind the male pheromones 2-(*sec*-butyl)thiazoline and dehydro-*exo*-brevicomin, and concentrate them in urine.
Key words. Pheromone; binding; proteins; urine; male mouse.

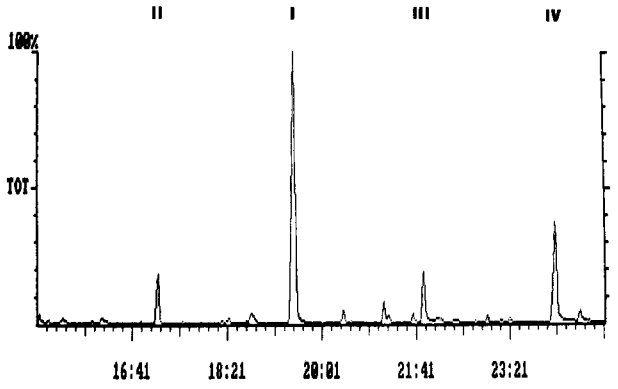
The ecology of the mouse is very dependent of olfactory cues. Chemical stimuli for intra-species communication (pheromones) are released with the urine by sexually mature – but not by impuberal nor castrated – male mice, and influence hormonally driven functions of female conspecifics, e.g., the induction of estrus (Whitten effect)[1] and the male-induced block of embryo implantation (Bruce effect)[2], as well as the behavior of conspecifics[3]. Some pheromones of the urine of the male mouse have been isolated and characterized; 2-(*sec*-butyl)thiazoline (**I**), 2,3-dehydro-*exo*-brevicomin (**II**), α- and β-farnesene[4,5]. The urine of the sexually mature – but neither impuberal nor castrated – male mouse is characterized by a high concentration of small proteins, the Major Urinary Protein Complex[6,7], whose function is not known. It seems probable that these proteins do have an important function, for three reasons. Firstly, the urinary loss of roughly 1 mg protein per day is a high cost for a mouse which in the wild state often ekes a life on the brink of starvation. Secondly, the genomic representation of the protein complex is highly amplified. Thirdly, the genes expressing the urinary proteins are highly conserved within the species, indicating a phylogenetic pressure against change in amino acid sequence[8]. An olfactory function seems plausible[9,10]. The present study showed that the proteins of the Major Urinary Complex selectively bind 2-(*sec*-butyl)thiazoline (**I**) and 2,3-dehydro-*exo*-brevicomin (**II**).

Methods and results

Major Urinary Proteins (MUP), separated from the very great variety of organic compounds present in urine by means of dialysis and chromatography, still retained bound pheromones. The gas chromatography and mass spectroscopy of the CH_2Cl_2 extract of purified MUP (fig.) showed almost exclusively pheromones **I, II**[11] and 4-(ethyl)phenol (**III**) in the ratio 17/3/4, and a non-identified compound (**IV**) with the mass spectrum of a terpene different from α- and β-farnesene. About 40% of the proteins had pheromone selectively bound to them, assuming complete extraction in CH_2Cl_2.

The approximate values of the binding constant of synthetic pheromones **I**, the two enantiomers (+)- and (−)-**II** and **III**, were assessed. The equilibrium binding of pheromones to the second isoform of MUP isolated by DEAE liquid chromatography from mouse and Wistar rat urine was studied in dialysis by displacement of 2-





Gas chromatogram of the CH_2Cl_2 extract of purified Major Urinary Proteins. The x-axis is time in minutes and seconds. Urine was collected from adult male albino mice (Swiss-NMRI) and MUP purified with a pressure dialysis of 15 h and a gel filtration. MUP were further resolved into the isoforms with DEAE ion exchange liquid chromatography[10]. MUP of the second isoform (20 mg/ml) were extracted with 0.2 vol of CH_2Cl_2. One µl of the organic phase, separated by sedimentation (1000 × g, 10 min) was analyzed by gas chromatography (SPB 1, 30 m, 0.32 mm diameter capillary column, split-splitless mode and PTV injector, injector temperature 260 °C, temperature program 8 min at 45 °C then 5 °C/min to 200 °C, carrier gas helium) and mass spectroscopy with an ion-trap mass-analyzer. The identification of the pheromones was by retention time in gas chromatograpphy and fragmentation in mass spectroscopy by comparison with synthetic samples. MS: *m/z* (**I**) 144(M^+, 2%), 128(26%), 115(100%), 84(7%), 60(90%), 59(49%); (**II**) 155(M + 1, 8%), 154(M^+, 10%), 136(5%), 125(43%), 111(98%), 95(100%), 87(8%), 83(54%), 81(51%), 67(39%), 57(53%), 53(43%); (**III**) 122(M^+, 32%), 107(100%), 91(5), 77(19%), 63(7%), 55(3%), 51(8%); (**IV**) 136(7%), 123(11%), 121(12%), 111(5%), 107(6%), 105(6%), 93(49%), 84(10%), 81(46%), 69(100%), 67(37%), 53(24%). **I** was synthesized as described for 2-(methyl)thiazoline in Fitton and Smalley[21], the two enantiomers (+)- and (−)-**II** were a gift of K. Mori and β-farnesene was obtained by dehydration of (E)-nerolidol[22].

isobutyl-3-methoxypyrazine (IBMP)[9]. Proteins were previously treated to remove natural ligands with mM IBMP, extracted with n-pentane and treated with activated charcoal. Aliquots of protein (20 nmol in 0.5 ml) were brought at equilibrium in cellulose dialysis bags (M_r cutoff 5000) with shaking at room temperature for 20 h. The external solutions were 20 ml of 10 M Tris/HCl buffer pH 8.3 with the test pheromone, IBMP and HPLC-purified (^{3}H)IBMP (1000 dpm/ml, 1.3 Ci/mM)[12]. Three concentrations of IBMP were used 0.25 µM, 0.5 µM and 1 µM for mouse MUP and 1 µM, 2 µM, and 4 µM for rat MUP, and 5 µM, 10 µM and 20 µM of the test pheromone. The inhibition constant K_i was determined from at least 18 experimental points with linear regression analysis of Scatchard plots and assuming competitive inhibition. The K_m for IBMP is also reported. The radioactivity inside the bag was at least three times the external radioactivity. The variability of repeated points was within 10% of the mean and that of the K_i about 30% of the mean. Controls were run with the solvent. **I** and **III** were stored in ethanol and **II** in n-pentane at −20 °C.

The binding constants are reported for mouse and rat MUP in the table. **I**, a molecule different from any other known pheromone, was bound with the greatest affinity. There was no difference between the two enantiomers of

Competition of synthetic pheromones **I**, (+)-**II**, (−)-**II** and **III** with 2-isobutyl-3-methoxypyrazine (IBMP) for binding to MUP of mouse and rat

Pheromone	K_i/µM	
	Mouse	Rat
I	10	5
(+)-**II**	20	9
(−)-**II**	21	11
III	27	> 30
IBMP	0.8	1.3

II. Interestingly, only the hydrogenated form of (+)-**II** is a pheromone for the insect *Dendroctonus brevicomis*[13]. The pheromone activity of **III** has not been determined up to now. The structure of **IV** is also an open question. Little is known about rat pheromones, but the binding suggests that mouse and rat share **I** and **II**. In general, the binding affinity is much lower than would be expected for a very stable binding controlled only by diffusion. A reaction may take place after binding to stabilize the pheromone-protein complex.

Discussion

The binding of sex pheromones **I** and **II** characterizes the proteins of the Major Urinary Complex as sex pheromone-binding proteins. Sex pheromone-binding proteins have been described in insect antennæ (see, e.g.[14, 15]) and in the saliva of the boar[16], but this is the first demonstration in rodents. This important function could explain the costly urinary protein loss, the gene amplification and the selective pressure to conserve the amino acid sequence. X-ray diffusion experiments on crystals of MUP with bound pheromone can now show the structure of the complex[17]. The binding of pheromones in this complex in the blood plasma could prevent their dispersal with breathing as well as reabsorption in kidney tubuli. Finally, the pheromones will be concentrated in the urine, where the normal MUP concentration is about 10^{-4} M. As urine is released in the field and dries, the pheromones might diffuse into the air and be sniffed by conspecifics. However, the volatility of **I** and **II** is rather low (b.p. 176 °C for **I** and 90 °C at 55 Torr for (+)-**II**[18]), so it is more likely that the protein-pheromone complex reaches the vomeronasal organ, where sex pheromones are known to be active[19], with licking. Whether pheromone-binding proteins also present the pheromone to receptor cells is still being debated[15]. In this context a highly conserved and positively charged motif in residues 28–32 of the urinary and nasal odorant binding proteins[20] could be a site for olfactory cell recognition.

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Correction I

2-Hydroxy-5-methyl-1,4-benzoquinone from the salivary gland of the soldier termites *Odontotermes magdalenae*

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Abstract. Attention is drawn to certain errors in our recently reported mass spectral study[1] of the salivary gland secretion from soldier termites of *Odontotermes magdalenae.*
Key words. 2-methyl-1,4-benzoquinone; 2-methyl-1,4-hydroquinone.

In this note, we present some corrections concerning a recently published combined gas chromatographic-mass spectral study of the salivary gland secretions from soldier termites of *Odontotermes magdalenae*[1].
Peak 1: There were some omissions in the mass spectrum data reported. The complete data should read: 92(M^+ 70), 91(100), 71(4), 74(5), 65(15), 63(9), 51(10), 43(14), 39(20), 30(12), 28(29).
Peak 2: The mass spectrum data reported is correct but should be for 2-methyl-1,4-benzoquinone and not the previously reported 1,4-benzoquinone (a typographical omission of the 2-methyl substituent).
Peak 5: The ion m/z 50(29) should read 150(29). This is a typographical error.
Peak 6: The mass spectrum data is correctly reported but should be for 2-methyl-1,4-hydroquinone instead of the previously reported 2-methyl-1,4-benzoquinone (another typographical error resulting from transposition of data from the original work[2]).

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Correction II

T. Watanabe and **R. M. Pratt:** Effects of retinoic acid on embryonic development of mice in culture; **Experientia 47 (1991) 493–497.** The micrographs in figure 1 of this paper were prepared by Dr. Barbara D. Abbott (currently with the United States Environmental Protection Agency) and should have been credited as such.