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Synthetic pheromones that promote inter-male aggression in mice

(chemical signals/behavior/olfactory message)

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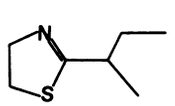
ABSTRACT **Two volatile constituents of male mouse urine, dehydro-*exo*-brevicomin and 2-(*sec*-butyl)-dihydrothiazole, have been found active in bioassays of inter-male aggressive behavior. The two synthetic compounds act synergistically when added to castrated male urine but not when added to water, and they provoke fighting that is quantitatively and qualitatively comparable to that elicited by intact male urine.**

Chemical communication regulates social behavior and reproductive physiology of *Mus musculus*, the common house mouse (1, 2). Among the important chemical signals described for this species is an androgen-dependent pheromone that provokes fighting attacks among adult males. Intact stimulus animals elicit a stereotyped sequence of sniffing and aggressive arousal that culminates in biting attacks and chasing. In contrast, castrated stimulus animals receive little aggression from normal trained "fighter" males (3). However, castrated stimulus animals anointed with urine from an intact male elicit more attacks than similar animals coated with urine from a castrated male (4, 5).

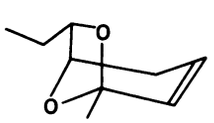
Although male bladder urine has this aggression-inducing property, externally voided urine might be more potent (6, 7), suggesting that other chemical cues, such as secretions from the preputial glands, may add to the biological activity of urinary compounds. While urine from intact females decreases aggression in male mice, the urine of testosterone-treated females is effective in inducing male aggression (5, 8–10), emphasizing the important relationship between androgens and this biological signal.

Past attempts to chemically characterize the male aggression pheromone have been unsuccessful. While some fractionation of the aggression activity into different organic solvents was previously observed (6), precise identification was obscured by numerous endogenous metabolites excreted into the mouse urine.

During our recent systematic chemical characterization of urinary volatile components in *Mus musculus* (11), we identified two substances that have clear dependence on testosterone levels (11); these are 2-(*sec*-butyl)-dihydrothiazole (compound **I**) and dehydro-*exo*-brevicomin (compound **II**).



2-*sec*-butyl dihydrothiazole



dehydro-*exo*-brevicomin

These compounds are found in the normal male mouse; castration drastically reduces their concentration in urine, while testosterone supplementation [at least for dehydro-*exo*-brevicomin (12)] renews the normal amounts. Both compounds have been synthesized (13, 14). We now report that the behavioral activity of these compounds together, but not alone, closely resembles that of normal male urine. Urine from castrated mice, spiked with compounds **I** and **II**, becomes an aggression-inducing signal. Spiked water does not.

Adult C57/BL male mice were used as test animals because their aggressive behavior parameters are well established and stable (15). On the basis of our past work on chemical communication in *Mus musculus* (12, 13, 16), we are most experienced with the levels of urinary volatiles in the BALB/cWt strain and, therefore, such stock (The Jackson Laboratory) was used as the source of urine for these experiments. Both normal and castrated males between 2 and 6 months of age served as urine donors. Dehydro-*exo*-brevicomin and 2-(*sec*-butyl)-dihydrothiazole, synthesized in our laboratory, were spiked into urine of castrated mice at concentrations simulating their content in normal urine (≈1.3 ppm; vol/vol). Because both compounds possess optical activity, and we synthesized them as racemic mixtures, the concentration of spiked components was intentionally doubled for testing in the aggression assay.

Aggression testing took place in the home cages of isolated fighters. After the bedding had been changed, 48 hr was allowed before testing was resumed. Trials consisted of 4-min presentations of a stimulus animal into the fighter's cage. The number of bites, cumulative attack time, and latency to first attack were measured as aggression parameters (4, 5, 7, 8). An untreated castrate was used for the first introduction. After 40 min, the same castrate was painted (0.03 ml) on the back and abdomen with a test substance or a control solution and placed with the fighter (second introduction). The same aggression parameters were monitored during the 4-min trial. The castrated animals were allowed at least 2 days between tests, in order to recover and allow any test substance to dissipate. Tests were conducted between 3:00 and 5:00 p.m.

The following solutions were tested in this bioassay for aggression: (*i*) normal male urine; (*ii*) castrated male urine; (*iii*) both compounds **I** and **II** spiked into castrated male urine; (*iv*) either compound **I** or **II**, spiked individually into castrated male urine; and (*v*) both compounds **I** and **II**, spiked at appropriate concentrations into distilled water. In various experimental arrangements and combinations, normal male urine and water were used to establish baseline control levels of the extreme situations—i.e., as the most potent and the least potent source of an aggression signal. Ten "experienced fighters" were divided into three groups. When testing started, one group received a test solution, while the other groups received control solutions (whole urine or water). Treatments were rotated and counterbalanced among the groups so that after 3 days of testing, each group had been tested with both controls and the test solution. Testing continued in this manner until 30 trials

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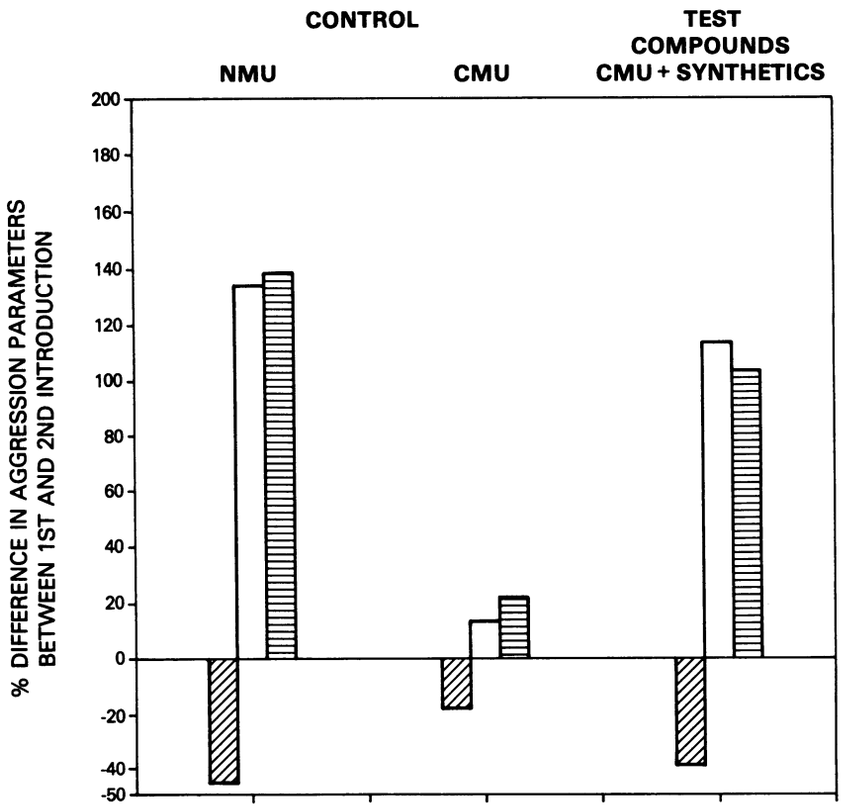

FIG. 1. Percentage difference in aggression parameters between first and second introduction. Diagonal stripes represent latency to first bite, clear blocks represent cumulative attack time, and horizontal stripes represent number of bites. NMU, normal male urine; CMU, castrated male urine.

were conducted. Several test sessions were videotaped for slow-motion analysis of behavioral patterns.

The Wilcoxon matched-pairs signed-ranks test was used for data reduction. One-tailed probabilities of 0.05 or less were considered significant. First, differences between the first and second introductions were calculated for the aggression parameters. Then, differences between difference scores were subject to analysis by the Wilcoxon test.

In agreement with observations by others (5, 6), our procedure verified that castrates painted with normal male urine were attacked more vigorously than castrates painted with urine from castrates. Both cumulative attack time and number of bites recorded for normal male urine and castrated male urine spiked with compounds I and II were significantly different from castrated male urine (Wilcoxon, $P < 0.05$). There is a trend toward shorter latency to first attack when normal or spiked urine is compared to the urine from castrated donors; however, differences in this parameter were not judged to be statistically significant.

Through plotting the percentage difference in aggression parameters (second introduction mean minus first introduction mean divided by first introduction mean $\times$ 100), we present our data graphically in Fig. 1. It is evident that urine from castrated animals spiked with both substances approaches the effectiveness of normal male urine in eliciting aggression for all three aggression parameters.

Videotapes of fighting sequences induced by intact urine and spiked urine from castrated mice were scrutinized during slow-motion playback. The synthesized stimulus elicited fully elaborated sequences of aggressive behavior. The qualitative similarities were striking. The synthetic stimulus appeared to communicate an olfactory message similar to the biological stimulus, as evidenced by the responses of the resident mice.

In similar experiments with the two synthetic substances spiked into water, or either compound I or II spiked into castrated mouse urine, no significant increase of aggressive behavior was noted. Fighter males did not demonstrate a significant increase in attack times over castrates painted with these solutions than those painted with water alone.

The fact that neither compound is active when spiked separately into castrate urine suggests that these chemicals act synergistically to elicit aggression. The results further indicate that compounds I and II are effective only if perceived in the context of the general odor of mouse urine. This is consistent with the view of Evans *et al.* (17) that "the Gestalt of the whole urine" is responsible for the aggression-eliciting property of normal male urine.

Some investigators (6–8) maintain that the preputial glands may be a source of aggression-inducing chemical signals. We have chromatographically compared the purgeable volatiles from both bladder urine and externally voided urine to see if there is a difference in concentrations of compounds I and II. As the results indicated comparable concentrations in both types of urine, preputial glands are an unlikely source of these compounds. The possibility, however, still exists that additional compounds of preputial origin may further add to the aggression-promoting properties of normal male urine.

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