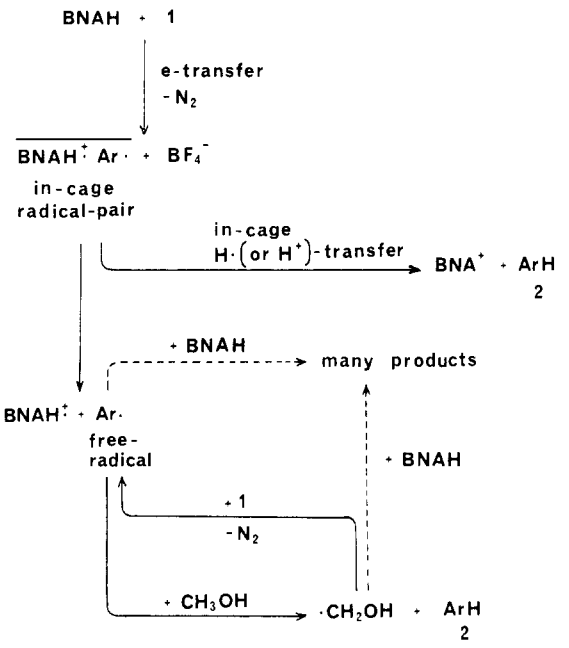
Scheme I



the ordinary method prior to use. Deuterated methanols were commercially available (Commissariat a l'Energy Atomique).

General Procedures. To 6.0×10^{-2} mmol of **1** was added a solution of an appropriate amount of BNAH in 3 mL (for **1a,b**) or 5 mL (for **1c**) of methanol. The reaction mixture was stirred for 10 min in the dark at room temperature under a nitrogen or oxygen atmosphere, and the products were analyzed. The reduction product **2** was analyzed as follows: a 0.25-mL aliquot of the reaction mixture was quenched with 1 mL of water and extracted with 1 mL of ether containing an internal standard for VPC analyses (*p*-xylene, *p*-methylanisole, and *m*-dimethoxybenzene, for **2a-c**, respectively). Then, the organic layer was analyzed on a Yanaco G-180 gas chromatograph (SE-30 column). The deuterium content in **2** from the reaction in deuterated methanols was determined by using a Hewlett Packard 5992B GC/MS spectrometer.

In order to determine the amount of **1c** recovered, a diazo coupling reaction was employed: to 5 mL of water was added 0.5 mL of the reaction mixture, and the aqueous layer was washed twice with 4-mL portions of dichloromethane. A 100-μL aliquot of the aqueous layer was added to a solution with 1.2×10^{-2} mmol of 2-naphthol in 3 mL of ethanol in a UV cell, and the absorbance of the 1-[*p*-(nitrophenyl)azo]-2-naphthol formed was measured on a Hitachi 220 spectrophotometer at 482 nm and compared with that obtained from a reference run (in which BNAH was absent). The spectrum in the reference run was consistent with that of the authentic sample. It was also confirmed that the observed absorbance obeys Beer's law within the range of the concentrations employed.

Moreover, 9.0×10^{-1} mmol of **1a** was allowed to react with 1.8 mmol of BNAH in 45 mL of methanol, with stirring in the dark at room temperature, under a nitrogen atmosphere. After 30 min, the reaction mixture was poured into 50 mL of water and extracted twice with dichloromethane. The organic layer was washed twice with water saturated by sodium chloride, dried over anhydrous sodium sulfate, and concentrated in vacuo. The residue gave many spots on TLC. The mixture was subjected to separation with preparative TLC first with benzene eluenet and second with ethyl acetate eluent. All fractions were analyzed on a JASCO JNM-FX 100 FT NMR spectrometer, but only one (<4 mg, <2%) was identified, as *p,p'*-dimethylbiphenyl. One of the products gave an NMR spectrum ascribable to the structure of *N*-benzyl-6-(4'-methylphenyl)-1,4,5,6-tetrahydronicotinamide. However, a small amount of contaminat(s) prevented us from identifying it. The other products could not be identified at all.

Registry No. **1a**, 459-44-9; **1b**, 673-40-5; **1c**, 456-27-9; BNAH, 952-92-1; NADH, 58-68-4.

Structural Determination and Synthesis of a Chemical Signal of the Male State and a Potential Multipurpose Pheromone of the Mouse *Mus musculus*

Donald P. Wiesler, Frank J. Schwende, Marvin Carmack, and Milos Novotny*

Department of Chemistry, Indiana University, Bloomington, Indiana 47405

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exo-7-Ethyl-5-methyl-6,8-dioxabicyclo[3.2.1.]oct-3-ene (1) has been isolated from urine of the male mouse of the species *Mus musculus*. It is one of a small number of volatile constituents that signal the adult male state and induce intermale aggression. It may also be regarded as a potential multipurpose male mouse pheromone. Its structure elucidation and synthesis are described.

Chemical constituents in the urine of adult male mice have long been known to produce diverse behavioral and endocrine responses in both female and male mice.[1-3] During a detailed investigation[4,5] of volatile urinary constituents by high-resolution, gas-phase analytical techniques (capillary gas chromatography, combined with both low- and high-resolution mass spectrometry and Fourier-transform infrared spectroscopy), we have focused attention especially on those compounds that were clearly associated with the masculinity of this animal species. While the mouse urine samples contain well over 100 volatile components,[4,5] only a small number of these (less than five) show clear dependency on the male hormone, testosterone. These may be suspected to be potential male pheromones.

One compound in particular stood out as an indicator of the adult male state: it was practically absent in both females and castrated males, but its normal urinary concentration was readily renewed through treatment with testosterone.[6] We have now identified the structure of

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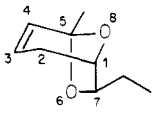
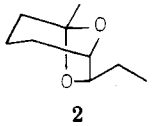
the compound as 1 (*exo*-7-ethyl-5-methyl-6,8-dioxabicy-



1



2

clo[3.2.1]oct-3-ene) on the basis of spectral properties, chromatographic retention, and finally synthesis, followed by catalytic hydrogenation to the known compound *exo*-brevicomin (**2**). The latter has been previously identified as a pheromone of the western pine bark beetle *Dendroctonus brevicomis* and some other insect species.[7,8] Although olefinic analogues of **2** have apparently not been found among insect pheromones, the occurrence of the structurally similar bridged acetals **1** and **2** in such widely separated branches of the animal kingdom is of special interest.

While extensive biological testing of **1** for a variety of primer and behavioral effects is being pursued in our laboratory, our recent data establish[9] that **1**, in combination with another uniquely male mouse compound, 2-*sec*-butyl-4,5-dihydrothiazole,[10] is an aggression-promoting principle of the adult male mouse.

We were able to deduce the correct structure of **1** from Fourier-transform infrared and mass spectral data. This was possible at a stage where quantities of purified **1** from the high-resolution analytical glass capillary gas chromatographic procedures were too minute to permit reliable NMR analysis. The precise molecular weight of 154.0986 corresponds to the molecular formula $C_9H_{14}O_2$. The relatively short retention time for a compound of that molecular weight, containing two oxygen atoms, considered together with the absence of absorption peaks in the carbonyl and hydroxyl stretch regions of the IR spectrum, suggested a compact molecule with two ether functions. Comparison with a large number of published IR spectra of ethers[11] revealed the unlikelihood of either oxygen atom's being part of an epoxide or vinyl ether moiety while subtly suggesting a ketal unit.

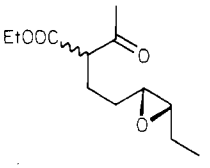
A peak in the mass spectrum at *m/e* 96 (M − 58, C_6H_8O), along with evidence for the facile loss of an ethyl, but not a methyl, group upon electron impact suggested that any proposed structure should permit ready expulsion of a propanal unit. The intense peaks at *m/e* 43 and 111 (M − 43, $C_7H_{11}O$) suggested the $-OC(CH_3)$ grouping. Infrared evidence pointed to a cis-disubstituted carbon–carbon double bond.

Many similarities between the IR spectra of **1** and **2**[12] were noted, but the mass spectrum of **2**[13] contained a much more intense peak at *m/e* 43; a carbon–carbon double bond was therefore tentatively assigned to the position C_3 as shown in **1** in order to explain the less facile cleavage at the ketal carbon atom (C_5) in comparison with **2**.

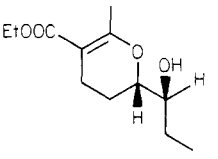
The olefin **1** has been previously described as the product of an unusual photochemical sequence starting from methyl vinyl ketone dimer (in 14% overall yield).[14] We considered it essential to develop a straightforward synthesis that would not only reliably confirm the tentative structure **1** but also permit the preparation of larger quantities for experiments in animals, including structural variants to establish activity relationships.

Our synthesis of **1** using ethyl acetoacetate embodies some of the features of the synthesis of *exo*-brevicomin by Rodin et al.[15] but allows for the introduction of the olefinic function and various structural modifications.

Commercially available *cis*-3-hexen-1-ol was converted in a standard manner to its tosylate **3**, which in turn was converted to 1-iodo-3,4-*cis*-epoxyhexane (**4**) with sodium iodide in acetone. The iodide **4** was found to give superior results to **3** in the next step: alkylation of ethyl sodioacetoacetate to yield the dihydropyran **6**. The presumed intermediate, **5**, was not isolated. The dihydropyran **6** appears to be a single pure racemate and is a convenient holding point in the synthetic sequence.[16]

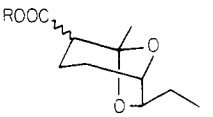


5



6

The dihydropyran **6** undergoes internal conjugate addition in the presence of boron trifluoride etherate to yield the saturated ester **7a**. The assignment of the (±)-exo



7a, R = Et
b, R = H

stereochemistry to **7a** follows from its subsequent two-step conversion into **1**, and the catalytic hydrogenation of **1** to **2**. The rearrangement thus follows a course analogous to the example of Sum and Weiler.[17]

The chromatographically purified **7a** was saponified and converted into its free acid **7b**, which reacted with lead tetraacetate in the presence of cupric acetate and pyridine with the loss of the carboxyl function and formation of the carbon–carbon double bond. It was desirable to purify the intermediate **7a** and the product **1** by column chromatography; other intermediates could be carried forward without special purification. Purification of **1** by chromatography on a column of silica gel pentane–ether (94:6) produced **1** in 37% overall yield from *cis*-3-hexen-1-ol.[18]

The catalytic hydrogenation of **1** to **2**, which established the structure and stereochemistry, was carried out as described by Chaquin et al.[14] to yield (±)-*exo*-brevicomin identical with the product described by Silverstein et al.[7]

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(18) Another variant of the above synthesis was explored. The use of $PhSCH_2COCH_3$ instead of ethyl acetoacetate, followed by oxidation of the thio ether to a sulfoxide, made use of the elimination of the phenylsulfinyl group to generate the olefinic function. Neither the alkylation nor the elimination step in this case proceeded to as satisfactory a degree as in the previously discussed case, however.

Experimental Section

Infrared spectra (IR) of liquids were determined on a Perkin-Elmer Model 298 spectrometer. Proton magnetic resonance (^{1}H NMR) spectra were obtained on a Varian EM-390 spectrometer. Mass spectra were determined on a Hewlett-Packard 5992A gas chromatograph/mass spectrometer (GCMS). Reactions were routinely carried out under a nitrogen atmosphere. Lead tetraacetate was prepared shortly before use. Microanalyses were performed by Galbraith Laboratories, Inc., Knoxville, TN.

(*Z*)-2-Ethyl-3-(2-iodoethyl)oxirane (4). The epoxy tosylate (9.25 g, 34.3 mmol), prepared by the action of *m*-chloroperbenzoic acid upon the tosylate of (*Z*)-3-hexen-1-ol (Alfa) in quantitative yield, was stirred for 3 h in 60 mL of acetone saturated with sodium iodide. After the mixture had been partially concentrated, the residue was taken up in ether and washed with aqueous sodium thiosulfate and brine and dried over anhydrous magnesium sulfate. Concentration yielded 6.92 g (30.6 mmol, 92%) of essentially pure **4** as judged by TLC. Further purification was achieved by Kugelrohr distillation: bp 69–71 °C (1.0 mm); ^{1}H NMR ($CDCl_3$) δ 3.26 (t, 2 H), 2.7–3.2 (m, 2 H), 1.8–2.2 (m, 2 H), 1.50 (dt, 2 H), 1.03 (t, 3 H); IR (neat) 1250, 1180, 940, 900, 810 cm^{-1}; mass spectrum (70 eV), *m/e* (relative intensity) 226 (M^+, 4), 168 (11), 81 (13), 57 (26), 43 (100). Anal. Calcd for $C_6H_{11}IO$: C, 31.88; H, 4.91; I, 56.14. Found: C, 31.92; H, 4.85; I, 55.86.

Ethyl 3,4-Dihydro-2-(1-hydroxypropyl)-6-methyl-2*H*-pyran-5-carboxylate (6). To 8.61 mmol of sodium ethoxide (from 198 mg of sodium) in 7 mL of absolute ethanol was added 1.10 mL (1.12 g, 8.64 mmol) of ethyl acetoacetate. After 15 min, 1.62 g (7.19 mmol) of epoxy iodide **4** in 2 mL of ethanol was added and the mixture heated under reflux for 18 h. After most of the solvent had been distilled, water was added and an ether extract was washed with brine and dried over anhydrous magnesium sulfate. Concentration gave 1.64 g (7.19 mmol, 100%) of **6**, shown by TLC to be pure: IR (neat) 3580 (OH), 1695 (C=O), 1625 (OC=C), 1245, 1060 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 4.17 (q, 2 H), 3.3–3.9 (m, 2 H), 2.0–2.8 (m, 2 H), 2.24 (s, 3 H), 1.3–2.0 (m, 4 H), 1.27 (t, 3 H), 1.00 (t, 3 H); mass spectrum (70 eV), *m/e* (relative intensity) 228 (M^+, 11), 186 (14), 183 (21), 131 (47), 98 (45), 97 (32), 43 (100). Anal. Calcd for $C_{12}H_{20}O_4$: C, 63.13; H, 8.83. Found: C, 62.87; H, 8.68.

Ethyl 7-Ethyl-5-methyl-6,8-dioxabicyclo[3.2.1]octane-4-carboxylate (7a). A solution of 568 mg (2.49 mmol) of the dihydropyran **6** in 15 mL of methylene chloride was stirred at room temperature for 2 h with 1.6 mL of freshly distilled boron trifluoride etherate. The resulting mixture was divided between water and ether. The ether layer was washed with brine and aqueous sodium bicarbonate and dried over anhydrous magnesium sulfate. Concentration gave 522 mg of a mixture consisting almost entirely of two diastereomers **7a** as determined by TLC and NMR. After chromatography on Merck 60H silica gel from pentane/ether (80:20) solution, this diastereomeric mixture was obtained in 75% yield based on epoxy iodide **4**: IR (neat) 1730 (C=O), 1160 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.9–4.4 (m, 4 H), 2.4–2.8 (m, 1 H), 1.4–2.2 (m, 6 H), 1.52 and 1.49 (pair of singlets in ca. 2:5 ratio, 3 H), 1.27 and 1.25 (pair of triplets, 3 H), 0.88 (t, 3 H); mass spectrum (70 eV), *m/e* (relative intensity) 228 (M^+, 1), 186 (54), 183 (17), 140 (29), 112 (20), 95 (19), 86 (17), 68 (23), 43 (100). Anal. Calcd for $C_{12}H_{20}O_4$: C, 63.13; H, 8.83. Found: C, 62.93; H, 8.95.

7-*exo*-Ethyl-5-methyl-6,8-dioxabicyclo[3.2.1]oct-3-ene (1). The chromatographed mixture of esters **7a** (1.51 g, 6.61 mmol) in 11 mL of ethanol was heated with 9 mL of 3 M sodium hydroxide under reflux for 7 h. The mixture was stirred at room temperature overnight, after which excess solvent was distilled. The neutral material was extracted with ether, after which the aqueous solution was acidified with 3 M hydrochloric acid, and the acid product **7b** was extracted with three portions of ether totaling 200 mL. The ether extracts were washed with brine, dried over anhydrous magnesium sulfate, and concentrated, yielding 1.29 g (6.45 mmol, 98% crude) of acid **7b**. Without further purification, the acid was mixed with 36 mL of benzene, 180 μL (0.18 g, 2.2 mmol) of pyridine, 310 mg (1.5 mmol) of cupric acetate monohydrate, and 5.2 g (11.7 mmol) of freshly prepared[19] lead tetraacetate. The mixture was heated for 2 h under reflux. An ether extract was washed with brine and aqueous sodium bicarbonate and then dried over anhydrous magnesium sulfate. The product **1** was chromatographed by using pentane/ether (94:6) on a column of Merck 60H silica gel. The yield was 554 mg (3.59 mmol), 54% based upon the diastereomeric ester mixture **7a**). It was homogeneous by GC and TLC: IR (neat) 2910, 1375, 1250, 1190, 1010, 960, 850 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 5.6–5.9 (m, 2 H), 4.24 (d, J = 4 Hz, 1 H), 3.78 (dt, 1 H), 2.1–2.8 (m, 2 H), 1.2–2.0 (m, 2 H), 1.50 (s, 3 H), 0.92 (t, 3 H); mass spectrum (70 eV), *m/e* (relative intensity) 154 (M^+, 4), 125 (14), 111 (32), 96 (14), 95 (22), 83 (17), 81 (12), 57 (21), 43 (100). Chaquin et al.[14] have reported IR and NMR data for their photosynthetic product.

The overall yield of purified **1** from (*Z*)-3-hexen-1-ol was 37%.

Acknowledgment. This research was supported by grant no. PHS R01 HD 14889 from the Institute of Child Health and Human Development, Department of Health and Human Services, U.S. Public Health Service. We thank Professor Robert M. Silverstein, SUNY, Syracuse, NY, for providing us with a sample of authentic (±)-*exo*-brevicomin. We are indebted to Dr. A. Fehl of the Procter and Gamble Research Laboratories, Cincinnati, OH, for providing us the use of capillary GC/FTIR spectrometer and to Drs. R. Milberg and J. C. Cook, Department of Chemistry, University of Illinois, for supplying us high-resolution mass spectra.

Registry No. 1, 88525-42-2; 4, 88525-43-3; 6, 88525-44-4; *endo*-7a, 88525-45-5; *exo*-7a, 88586-91-8; *endo*-7b, 88525-46-6; *exo*-7b, 88586-92-9; (*Z*)-3-hexen-1-ol tosylate, 34019-85-7; (*Z*)-3-hexen-1-ol epoxy tosylate, 88525-47-7; ethyl acetoacetate, 141-97-9.

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