A Novel Class of Defensive Compounds in Harvestmen: Hydroxy-γ-Lactones from the Phalangiid *Egaenus convexus*

Günther Raspotnig, V, Felix Anderl, V, Olaf Kunert, Miriam Schaider, Adrian Brückner, Mario Schubert, Stefan Dötterl, Roman Fuchs, and Hans-Jörg Leis

**ABSTRACT:** When threatened, the harvestman *Egaenus convexus* (Opiliones: Phalangiidae) ejects a secretion against offenders. The secretion originates from large prosomal scent glands and is mainly composed of two isomers of 4-hydroxy-5-octyl-4,5-dihydro-3H-furan-2-one (1), a β-hydroxy-γ-lactone. The compounds were characterized by GC-MS of their microreaction derivatives, HRMS, and NMR. After the synthesis of all four possible isomers of I, followed by their separation by chiral-phase GC, the absolute configurations of the lactones in the *Egaenus* secretion was found to be (4S,SR)-1 (90%) and (4S,SS)-1 (10%). Hydroxy-γ-lactones represent a new class of exocrine defense compounds in harvestmen.

Chemical defense in harvestmen (arachnid order Opiliones) is associated with large prosomal scent glands1 from which these arachnids discharge repellent secretions against predators.2 Exudates from these glands have been studied for more than 50 years,3 representing a rich source of rare, unusual, and new natural products. Current knowledge indicates that scent gland exudates comprise compounds such as naphthoquinones, chloro-naphthoquinones, and aliphatic methyl ketones in the harvestman suborder Cyphophthalmi, nitrogen-containing substances, terpenes, aliphatic ketones, and phenolics in the suborder Insidiatores, alkylated phenolics and benzoquinones in the suborder Grassatores, and secretions of rather miscellaneous chemistry in the Palpatores.2,4,5 While the latter group, the Palpatores, is subdivided into suborders Eupnoi and Dyspnoi1 and includes more than 2100 species of the Phalangiidae, the Palpatores is subdivided into suborders GC, the absolute configuration of the lactones in the *Egaenus* secretion was found to be (4S,SR)-1 (90%) and (4S,SS)-1 (10%). Hydroxy-γ-lactones represent a new class of exocrine defense compounds in harvestmen.

We focus here on the secretions of a first representative of the phalangiid subfamily Opilioninae, *Egaenus convexus*, a massively built species that is widespread in Central and Southeastern Europe. When threatened, individuals of *E. convexus* eject a “jet” from scent glands toward offenders (Figure 1).

Individual secretions directly dabbed from gland openings of adults of both sexes constantly showed two major peaks (A, B) by GC-MS. The peaks exhibited nearly identical EI-mass spectra, indicating isomeric compounds, with an earlier-eluting major isomer or mixture of isomers (peak A: RI = 1878 on a ZB-5MS column) and a later-eluting minor isomer or mixture of isomers (peak B: RI = 1901). Peaks A and B were present in a ratio of about 9:1 (Figure 2).

A molecular ion for these compounds was very weak in EI-MS but confirmed to be at m/z 214 by PCI-MS (positive ion chemical ionization; using methane as reagent gas: MH+ at m/z 215; M + C2H5 at m/z 243; M + C3H7 at m/z 255). HRESI-MS revealed an exact monoisotopic mass for [M + H]+ at m/z 215.1642, corresponding to a molecular formula of C12H22O3 and thus two sites of unsaturation/rings, respectively.

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Figure 1. (A) A female individual with the position of the ozopores (gland openings) indicated (arrows). (B) Ozopores (right ozopore is shown: arrow) are located near-dorsal to the coxa of leg I, are oval-shaped with a dimension of about 340 μm × 150 μm, and are surrounded by a cuticular rim (C, D). The flat central bottom is of smooth cuticle, whitish, thin, and membrane-like in appearance. This bottom is movable and trapdoor-like, and secretion can be released through a slit (D). Details of the right ozopore: (B) light microscopic photograph; note the whitish and membranous structure of the bottom of the pore; (C, D) scanning electron micrographs; note that the pore is slightly opened (arrow in (D)).

Figure 2. Total ion chromatogram of scent gland secretion of an individual of *Egaenus convexus*, showing main peaks A and B, and their EI-mass spectra (a, b). The secretion was dabbed from ozopores in the moment of extrusion (compare Figure S23).
Silylation of the extract with MSTFA (N-methyl-N-(trimethylsilyl)trifluoroacetamide) led to the TMS (trimethylsilyl)-derivatives 2 of the original compounds (Scheme 1). EI-MS of the original compound 1 as well as the fragmentations of the TMS-ether 2 were consistent with the structure of isomeric β-hydroxy-γ-alkyl-lactones. In detail, a β-hydroxy-γ-octyl lactone was supported by (i) characteristic fragment ions from the rearrangement of the lactone leading to m/z 143/142 (= furan oxygen plus an octyl group: Me(CH₂)₇CHO⁺ and Me(CH₂)₇CH₂O⁺, respectively); (ii) ions at m/z 124/125 (elimination of H₂O from the latter), together with (iii) m/z 44 (= OH-bearing moiety of the lactone ring: C₄H₄O⁺). In the TMS derivative 2, the corresponding fragment ions were recorded at m/z 116 (44 + 72: C₆H₁₀OSi) and m/z 215 (143 + 72: Me(CH₂)₇CHO⁺). The latter ion at m/z 215 arises by rearrangement of 3-O-TMS-alkano-4-lactones, supporting the octyl group and the OH group in the γ- and β-positions on the lactone ring, respectively. With this information given, the mass spectra of the remaining microderivatives 3–8 were interpreted (Scheme 1), finding full accordance with a 4-hydroxy-5-octyl-4,5-dihydro-3H-furan-2-one structure: saponification of 1 led to the opening of the lactone ring and to the generation of a 3,4-dihydroxydodecanoic acid (4), which could be converted into trimethylsilyl 3,4-bis((trimethylsilyl)oxy)dodecanoate (5) by methylation and silylation. On the other hand, the reduction of 1 led to lactone-ring opening and to the generation of 1,3,4-dodecanetriol (7), which could be converted into 1,3,4-tris((trimethylsilyl)oxy)dodecane (8) by silylation. Thus, peaks A and B were proposed to be isomers or mixtures of isomers of 4-hydroxy-5-octyl-4,5-dihydro-3H-furan-2-one (1).

Because the quantity of 1 in extracts of single individuals was too low to perform NMR analyses, a pooled extract containing the secretions of 400 individuals of both sexes was prepared. The major isomer of 1 from the pooled extract (corresponding to peak A in the chromatograms) was purified by column chromatography, followed by preparative gas chromatography. One dimensional 1H NMR of 1 (Figure 3; Table 1) fully supported the proposed hydroxy-lactone structure, revealing the presence of a linear alkyl (octyl) residue, two protons located on carbon atoms carrying oxygen substituents, and two diastereotopic protons present next to a carbonyl group.

A COSY spectrum indicated that these protons where located on adjacent carbon atoms (Figure S2). This is realized within a 5-membered lactone. Moreover, the observed chemical shifts and coupling constants were remarkably similar to the reported values for 4-hydroxy-5-methylbutyrolactone and 4-hydroxy-5-octylbutyrolactone in the literature. Additionally, a comparison to these γ-butyrolactones of defined configuration tentatively suggested that the major isomer of 1 was 4,5-anti-diastereomer, as indicated by coupling constants of the isolated H-3a and H-3b resonances (Figure 4).

Because 4-hydroxy-5-octyl-4,5-dihydro-3H-furan-2-one (1) possesses two stereogenic centers, four stereoisomers are possible. Two TMS moieties to two hydroxy groups. This is consistent with a methyl-ester bis-TMS-ether structure or a carboxyl group plus two hydroxy groups after saponification. Reduction of the original compounds using LiAlH₄ led to compound 7 with M⁺ at m/z 218, indicating the addition of four hydrogens, as confirmed by subsequent MSTFA derivatization (8: M⁺ at m/z 434; m/z 218 plus 3 × 72). By using LiAlD₄, two hydrogens and two deuterium atoms were added, resulting in a TMS product 8b of molecular weight at M = 436 g/mol (Scheme 1). Thus, the parent structure was indicated to carry two ketone/aldehyde functions with no ring or was a lactone.

The mass spectra showed the addition of a single TMS moiety to the original molecules (plus 72 mass units), with diagnostic ions at m/z 271 (M-1S) and a very weak molecular ion at m/z 286 (intensity < 1%). The TMS products were subsequently analyzed by PCI-MS, confirming the molecular ion at m/z 286 (MH⁺ at m/z 287, M + C₆H₁₂O⁺ at m/z 315). Accordingly, the derivatization with MTBSTFA (N-methyl-N-( tert-butylidimethylsilyl)trifluoroacetamide) indicated the addition of one butyldimethylsilyl group, leading to adduct 3, with M⁺ at m/z 328 (plus 114 mass units), as evidenced by PCI-MS (MH⁺ at m/z 329, along with C₆H₁₂O⁺ and C₆H₁₀O⁺ adds at m/z 357 and 369, respectively). Upon saponification (NaOMe), an addition of 18 mass units to the original compounds was observed (4: M⁺ at m/z 232). Subsequent derivatization with MSTFA led to compound 5 with M⁺ at m/z 448 (as confirmed by PCIMS), now showing the addition of three TMS moieties (232 plus 3 × 72 = 448). These data are consistent with three hydroxy groups or two hydroxy groups and one carboxyl group after saponification. Treatment with diazomethane following saponification and subsequent derivatization with MSTFA produced compound 6 with M⁺ at m/z 390, suggesting (i) the addition of a methyl group (to a carboxyl group) plus (ii) the addition of two TMS moieties to two hydroxy groups. This is consistent with a methyl-ester bis-TMS-ether structure or a carboxyl group plus two hydroxy groups after saponification.
The original extract showed two peaks (A, B) on an apolar nonchiral-phase column, indicating the presence of at least two (but potentially all four) stereoisomers of 1. To determine which of the isomers actually were present in the Egaenus extract and to elucidate their absolute configuration, all four stereoisomers of 1 were synthesized in enantiopure form.

Table 1. NMR Chemical Shift Values of the Authentic Sample and Corresponding Synthetic Compounds (4S,5R)-1/(4R,5S)-1 and (4S,5S)-1/(4R,5R)-1 with the Same Relative Configuration

<table>
<thead>
<tr>
<th>atom</th>
<th>authentic sample major component δ (J in Hz)</th>
<th>(4S,5R)-1/(4R,5S)-1 δ (J in Hz)</th>
<th>(4S,5S)-1/(4R,5R)-1 δ (J in Hz)</th>
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<tbody>
<tr>
<td>2</td>
<td>174.8, C</td>
<td>37.7, d, (17.9, 6.7)</td>
<td>37.7, d, (17.9, 6.7)</td>
</tr>
<tr>
<td>3</td>
<td>2.85 dd, (18.0, 3.8)</td>
<td>2.84, dd (17.9, 6.7)</td>
<td>2.80, dd (17.8, 5.6)</td>
</tr>
<tr>
<td>4</td>
<td>4.29, ddd (6.5, 4.0, 3.0)</td>
<td>4.28, ddd (6.5, 4.0, 3.0)</td>
<td>69.0, CH3</td>
</tr>
<tr>
<td>5</td>
<td>4.32, ddd (8.1, 5.4, 3.1)</td>
<td>4.34, ddd (8.1, 5.4, 3.1)</td>
<td>4.37, ddd (8.2, 5.7, 3.7)</td>
</tr>
<tr>
<td>1'</td>
<td>1.61-1.64, m</td>
<td>33.2, CH3, 1.64, m</td>
<td>28.3, CH3, 1.84, m</td>
</tr>
<tr>
<td>2'</td>
<td>1.29-1.44</td>
<td>25.2, CH3, 1.49, m</td>
<td>25.6, CH3, 1.50, m</td>
</tr>
<tr>
<td>3'</td>
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<td>29.2, CH3, 1.28, m</td>
<td>29.2, CH3, 1.29, m</td>
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<tr>
<td>4'</td>
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<td>29.3*, CH3, 1.28, m</td>
<td>29.5, CH3, 1.29, m</td>
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<tr>
<td>5'</td>
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<td>29.4*, CH3, 1.29, m</td>
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<tr>
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<tr>
<td>7'</td>
<td>1.29-1.44</td>
<td>22.7, CH3, 1.29, m</td>
<td>22.7, CH3, 1.27, m</td>
</tr>
<tr>
<td>8'</td>
<td>0.88, t (7.3)</td>
<td>14.1, CH3, 0.88, t (7.2)</td>
<td>14.1, CH3, 0.88, t (7.0)</td>
</tr>
</tbody>
</table>

*Resonances marked with an “*” could be either C-4' or C-5'. Data were recorded in CDCl3; TMS was used as the internal standard. J values are in Hz.

Figure 3. Comparison of the characteristic regions of the 1H NMR spectrum of the authentic sample with the same regions of the synthetic compounds. The resonances of the minor component in the authentic sample are marked with stars. The spectra were recorded in CDCl3 at 600 MHz for the authentic sample and at 700 MHz for the synthetic compounds (imp. = impurities).
form (Scheme 2; Supporting Information) and analyzed by NMR as well as by chiral-phase gas chromatography.

Both enantiomers of the syn diastereomer of 1 \([(4S,5S)-1\] and \((4S,5R)-1\)] were prepared in two steps from n-decanal according to an already described procedure. Knoevenagl-type condensation of n-decanal and monoethyl malonate yielded skipped ester 10. The Sharpless asymmetric dihydroxylation of this intermediate delivered both enantiomers of 1 in good yield and excellent optical purity. As the analogous route was not applicable to the corresponding anti-isomers \([(4S,5R)-1\] and \((4R,5S)-1\)] these were achieved in a different manner. Commercial enantiopure benzyl glycidyl ether was treated with n-heptylmagnesium bromide in the presence of catalytic amounts of copper salts to yield benzyl ether 11. TBS protection of the resulting hydroxy group led to compound 12, and the subsequent hydrogenolysis of the primary benzyl ether delivered monoprotected diol 13. TEMPO-catalyzed oxidation of the said alcohol provided aldehyde 14, which was subjected directly to an Evans aldol reaction with the boron enolate derived from 16. The ensuing product 15 was isolated as a single diastereomer in fair yield. Finally, the fluoride-mediated reaction provided the desired product 1.

Scheme 2. Synthesis of \((4S,5S)-1\) and \((4S,5R)-1^{a}\)

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**Chart 1. Stereogenic Centers and Possible Stereoisomers of 4-Hydroxy-5-octyl-4,5-dihydro-3H-furan-2-one (1)**

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**Figure 4.** Observed chemical shifts, multiplicities, and coupling constants of the major component 1 of the authentic sample (red) in comparison with previously reported values of synthesized γ-butyrolactones with a defined configuration (blue).
Lactones are quite frequent in nature and are well-known as scent/aroma compounds in fruits and milk and as flavoring compounds in alcoholic beverages (i.e., whiskey and cognac lactones). However, among arthropods, the γ-lactones found in the scent gland secretion of Egaenus are exceptional. First, they represent a novel class of compounds for the chemical inventory of scent gland secretions in harvestmen. Regarding the chemistry of Eupnoi, the compounds are similar to the acyclic compounds found in sclerosomatid Eupnoi. On the basis of our observations, the secretion of Egaenus also spreads over the body surface of jetting individuals, hence impregnating the body surface. Lactone amounts per individual were found to be highly variable, obviously depending on the filling status of the scent glands. In specimens extracted immediately after collection, we found 12.5 ± 7.9 μg/per individual with no obvious differences between the sexes. These amounts appear rather low, possibly indicating that the lactones are dissolved in a carrier matrix of currently unknown chemistry. In acyclic compound-producing leiobunines, this matrix is aqueous.

Considering secretion discharge following a disturbance, the mode of secretion application (i.e., a directed jet against offenders), and self-wetting of the body—surface, a defensive and antimicrobial role of the Egaenus secretion appears to be likely. These newly discovered lactones add an unexpected component to the overall picture of harvestmen-secretion chemistry. It will be a logical next step to investigate their chemical inventory of scent gland secretions in harvestmen. 

In contrast, the lactone motif itself is frequently present in exocrine exudates of arthropods as well as vertebrates. Lactones, for instance, are known from butterflies and beetles where they may serve as sex pheromones. Similar to large-ringed macrolides, γ- and δ-lactones have also been described as antimicrobial agents and a few additionally possess a role in predator defense.

Regarding harvestmen, scent gland exudates have generally been considered defensive, even though additional functions may have evolved in particular taxa. In some harvestmen species, chemical defense is indeed obvious: secretions are readily expelled as sprays or jets, reaching an offender at a distance of several centimeters, deterring invertebrates as well as small vertebrates. Concurringly, specimens of E. convexus forcefully eject secretion upon mechanical disturbance. This mode of secretion transfer is known from other harvestmen species and is called “jetting,” addressing the discharge of a fine, directed splash against offenders. So far, jetting has been mainly described for certain Laniatores whereas “spraying,” defined as a fine, vaporized spray, is known from a group of sclerosomatid Eupnoi. On the basis of our observations, the secretion of Egaenus also spreads over the body surface of jetting individuals, hence impregnating the body surface. Lactone amounts per individual were found to be highly variable, obviously depending on the filling status of the scent glands. In specimens extracted immediately after collection, we found 12.5 ± 7.9 μg/per individual with no obvious differences between the sexes. These amounts appear rather low, possibly indicating that the lactones are dissolved in a carrier matrix of currently unknown chemistry. In acyclic compound-producing leiobunines, this matrix is aqueous.
evolutionary origin and taxonomic distribution across the Opiliones.

**EXPERIMENTAL SECTION**

**General Experimental Procedures.** Optical rotations of compounds were measured using a Jasco P-2000 polarimeter at 25 °C. NMR spectra and mass spectra were recorded from solutions in CDCl3 (Bruker Biospin). NMRS spectroscopy was performed on either a Bruker Avance III HD 600 spectrometer or Bruker Avance III HD 700, respectively. Frozen traps from pGC (preparative capillary gas chromatography, see below) containing the purified authentic compound 1 were eluted with 500 μL of CDCl3, containing 0.03% TMS for reference (99.8 atom %D, Armar, Germany). Spectra were measured with either a cryoprobe at 298 K or with a quadrupole resonance probe (QXI HIS/13C/15N/31P) at 293 K. The final shift assignment was achieved with COSY and TOCSY spectra (120 ms mixing time). Raw data were processed in Topspin 3.2 (Bruker Biospin), and 2D data was analyzed using Sparky (T. D. Goddard and D. G. Kneller, SPARKY3, University of California, San Francisco). COSY, HSQC, and HMBC spectra of the synthetic compounds were recorded in 720 μL of CDCl3, at 25 °C with TMS as the internal standard on a Bruker Avance III HD 700 spectrometer. Data were processed with the MestReNova software package. Analytical GC-MS was performed on a Trace GC-DSQ I system (electron impact spectra; EI) and an ISQ Single Quadrupole mass spectrometer (positive ion chemical ionization (PCI), using methane as reagent gas; both systems were from Thermo Fisher). A list of all compounds is given in Table S4. All other chemicals were of reagent grade. Puriﬁcation was performed on silica gel (40–45 μm) for the separation of the crude extracts. The puriﬁcation of the pure synthetic compounds was performed on a Kieselgel 60 (0.5 mm, E. Merck) column. All solvents and reagents were obtained from ABCR, Carl Roth, and Sigma-Aldrich and were used as received unless stated otherwise.

**Preparation of Extracts.** 400 Adult individuals of both sexes of *Eugenea convexus* were collected by hand from May to July 2013 at the “Rosenhain,” Graz, Austria (N47.048838; E15.449741). An additional 55 individuals (from the same location) were collected in July 2019 and July 2020, respectively. All specimens were deposited in the collection of the Institute of Biology, Division of Zoology, University of Graz, Austria (voucher numbers RG 4240–4261, 4266–4275, 4277, 4288, 4325, 4349, 4356, 4365–4383, 4387, 4408–4411, 4416–4418, 4455, 4456, 4458, 4459, 5402–5411, 5414). A freshly emptied secretion was collected by dabbing the secretion on filter paper pieces (2 × 2 mm) directly from the gland openings (ozopores). “Loaded” filter papers were extracted in hexane (100 μL) for 15 min and gave extracts of pure secretion (Figure 2). Alternatively, individual whole-body extracts were prepared (500 μL of hexane, 15 min). The latter method was more feasible with respect to handling and resulted in equal or higher quantities of secretion per extract but showed additional, nonsecretion compounds in the extracts since some cuticular hydrocarbons were coextracted (Figures S24 and S25).

**Prepurification by Flash Chromatography.** Initial fractionation was performed on silica gel (40–63 μm) with solvents of ≥99% purity or p.a. grade. Hexane extracts, each containing the secretions of ~40 individuals (in ~10 mL), were concentrated in a stream of nitrogen to a volume of ~300 μL. Concentrated extracts were purified on silica gel columns packed with 500 mg of unmodiﬁed SiOH (Chromabond, 3 mL, Machery-Nagel) using solvents of ≥99% purity or p.a. grade. Purification of these solutions by flash chromatography (100% hexane → 100% CH3Cl) provided the compounds of interest in the CH3Cl fractions. In detail, columns were washed with six column equivalents (CE) of hexane before adding the extracts. Subsequently, the columns were eluted with 10 CE hexane to remove the cuticular hydrocarbons. Finally, the purified polar fraction was eluted with 10 CE CH3Cl. Fractions of 10 columns (corresponding to the secretion of 400 individuals) were combined and carefully concentrated in a stream of nitrogen. The residue was redissolved in hexane and subjected to preparative gas chromatography.

**Preparative Capillary Gas Chromatography (pGC).** The final purification of the major compound of the prepurified extracts (“peak A”) was accomplished by pGC using a preparative fraction collector (PFC). The GC-PFC system consisted of a gas chromatograph equipped with a flame ionization detector (FID: Agilent 7890A), a PFC device (Gerstel), and a ZB-5 fused silica capillary column (30 m × 0.32 mm ID, 0.25 μm) from Phenomenex. Hydrogen was used as carrier gas with a ﬂow rate of 3 mL/min. The column was split at the end by a pFlow splitter (Gerstel) into two columns leading to the FID (2 m × 0.15 mm ID) and the PFC (1 m × 0.2 mm ID), respectively. Nitrogen makeup gas with a flow rate of 25 mL/min was applied to the splitter. The PFC was connected with the GC oven via a heated transfer line, which was connected to seven transfer capillaries with an eight port zero-dead volume valve via the deactivated column (for further information about the setup, see refs 31 and 32). Four μL sample aliquots were injected via a multimode inlet (MMI) (Agilent) and heated to 320 °C. The temperature of the GC oven was raised from 40 to 270 °C with a heating rate of 25 °C per minute. The sampling time was 1 min, and the transfer line of the PFC was heated to 270 °C. The volatile traps were self-made microliter glass tubes ﬁlled with 50 mg of Carbrotab B (mesh 20–40, Supelco) and deactivated glass wool. The traps were ﬁxed in a handmade closed cylindrical glass pipe with a screw coupling with a sealing ring (SciLabware Ltd. Stone). The glass pipe with the trap used for fraction collection was placed in a self-made cooling block and chilled to −20 °C. After the preparative fractionation collection of the main compound (from 9.5 to 9.9 min), the traps were frozen at −20 °C until further processing.

**Compounds in Extracts.** 

(45R,5S)-4-Hydroxy-5-octyl-4,5-dihydro-3H-furan-2-one (45S,5R-1). *H NMR, Table 1. EIMS (70 eV): m/z 196 [M–18] (1), 143 (27), 142 (39), 125 (11), 124 (30), 115 (6), 111 (5), 102 (7), 101 (10), 98 (10), 97 (15), 96 (11), 95 (11), 93 (11), 89 (7), 88 (6), 84 (10), 83 (56), 82 (23), 81 (11), 71 (13), 69 (100), 68 (12), 67 (10), 57 (41), 55 (47), 44 (31), 43 (37), 41 (24). PCIMS m/z 225 [M + CH3H] (17), 243 [M + CH3H] (3), 215 [MH] (100), 197 (20), 195 (10), 179 (36), 161 (16), 155 (68), 137 (23). HRESIMS m/z 215.1642 [M + H]+ (calcd for C11H10O3, 215.1647), gas chromatographic retention index (ZB-5) 1878.

(45S,5S)-4-Hydroxy-5-octyl-4,5-dihydro-3H-furan-2-one (45S,5S-1). EIMS (70 eV): m/z 196 [M–18] (3), 143 (23), 142 (41), 136 (11), 131 (7), 125 (11), 124 (28), 115 (11), 112 (11), 111 (13), 110 (12), 102 (15), 101 (8), 98 (15), 97 (18), 96 (13), 95 (15), 89 (11), 84 (7).
Microderivatization. Aliquots of extracts were subjected to silylation, saponification, reduction, and methylation, respectively. For silylation, we used (i) MSTFA (N-methyl-N-(trimethylsilyl)-trifluoroacetamide in pyridine 2:1 containing 1% trimethylchlorosilane) and (ii) MTBSTFA (N-tert-butyldimethylsilyl-N-methyltrifluoracetamide containing 1% tert-butyldimethylchlorosilane), respectively. Saponification was performed using NaOCH₃ in MeOH; reduction was containing 1% TMCS, 2:1 in pyridine) was added, and the resulting mixture was incubated at 55 °C for 30 min, an aliquot of the mixture (1.5 μL) was directly used for GC-MS analysis. EIMS (70 eV) m/z 286 (<1), 271 (6), 229 (78), 227 (7), 215 (100), 143 (9), 129 (11), 117 (42), 116 (95), 101 (75), 75 (23), 45 (69), 65 (9), 59 (12), 55 (12), 43 (18), 41 (17). PCIMS m/z 327 [M + CH₃] (6), 315 [M + CH₄] (14), 287 [MH] (100), 269 (10), 229 (20), 215 (17), 197 (19), 179 (42), 161 (8), 137 (12).

Preparation of 4-Hydroxy-5-octyl-4,5-dihydro-3H-furan-2-one (1). Preparation of 5-Octyl-(trimethylsilyloxy)-dihydro-3H-furan-2-one (2). MSTFA (50 μL) was added to an aliquot of the scent gland extract in hexane (50 μL). The reaction mixture was incubated at 55 °C. After 30 min, an aliquot of the mixture (1.5 μL) was directly used for GC-MS analysis: EIMS (70 eV) m/z 271 [M − C₄H₉] (5), 230 (15), 229 (78), 192 (2), 147 (20), 133 (11), 116 (7), 103 (17), 83 (14), 73 (100). HRESIMS [M + H]⁺ (calcd 215.1647; gas chromatographic retention index (ZB-L) 301 (58), 285 (38), 248 (15), 215 (18), 165 (6).

**ASSOCIATED CONTENT**

**Supporting Information**

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jnatprod.0c00277.

Synthesis of reference materials; NMR spectra (¹H, COSY, and TOCSY spectra for authentic material, ¹H and ¹³C spectra for (4R,5S)-1, (4R,5R)-11, 12, 13, 15; COSY, HSQC, and HMBC spectra for (4R,5S)-1); El-mass spectra of microderivatives (compounds 2, 3, 5, 6, 8a, 8b); comparison of sampling techniques (direct sampling of secretion vs whole body extraction: total ion chromatograms and ¹H spectrum of whole-body extract) (PDF)

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**Notes**

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