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Note

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Polypropionates from the South African Marine Mollusk *Siphonaria oculus*

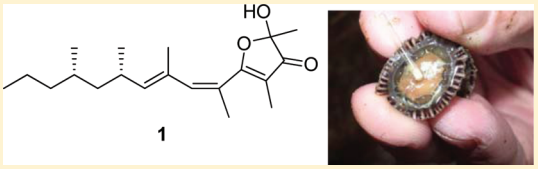
Candice L. Bromley,[†] Wendy L. Popplewell,[†] Shirley C. Pinchuck,[‡] Alan N. Hodgson,[‡] and Michael T. Davies-Coleman*[,†]

[†]Department of Chemistry, Rhodes University, Grahamstown, 6140, South Africa

[‡]Department of Zoology and Entomology, Rhodes University, Grahamstown, 6140, South Africa

Supporting Information

ABSTRACT: Three new polypropionate metabolites, 6*Z*,8*E*-Δ^8-siphonarienfuranone (**1**), 6*E*,8*E*-Δ^8-siphonarienfuranone (**2**), and 6*E*,8*E*-3-hydroxy-4,6,8,10,12-pentamethylpentadeca-6,8-dien-5-one (**3**), and the known polypropionate siphonarienfuranone (**4**) were isolated from the intertidal South African marine mollusk *Siphonaria oculus*. Evidence is presented to suggest that **1**, **2**, and **4** may cyclize from an acylic precursor on chromatographic workup of the acetone extract of this mollusk.



Marine mollusks of the genus *Siphonaria* (subclass: Pulmonata, family: Siphonariidae), commonly known as false limpets, are shelled, air-breathing herbivores that are believed to have a marine ancestry and are therefore considered as a possible evolutionary link between terrestrial and marine gastropod mollusks.[1,2] When disturbed, siphonariid limpets secrete a sticky white mucus from their lateral pedal glands (Figure 1).[2] While the mucus released by *Siphonaria*

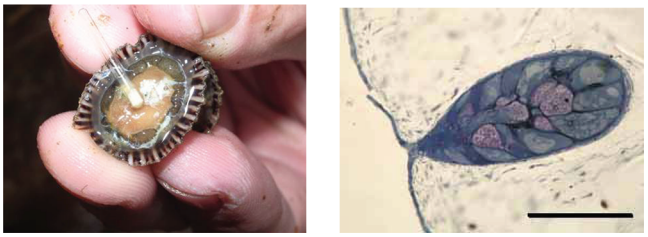


Figure 1. Collecting the mucus exudate from *Siphonaria oculus* (left). Light microscope image (section stained with toluidine blue) of a longtitudinal section through the lateral pedal gland of *S. oculus* (right). Scale bar corresponds to 0.1 mm.

species is rich in polypropionate secondary metabolites, which are believed to deter predators, the role of these polypropionates in siphonarid chemical defense has not been conclusively established.[2,3] The marine polypropionate research field was last reviewed in 1998 by Davies-Coleman and Garson[4] and later summarized for *Siphonaria* species *per se* according to the geographical distribution of members of this genus.[1,5,6] Despite the ubiquitous intertidal distribution of *Siphonaria* species, only three publications describing new polypropionate metabolites from this genus have been reported in the last 12 years.[7−9]

Colonies of *S. oculus* occur along the southern and eastern coast of South Africa and southern Mozambique, where they can be commonly found on sheltered rocks along the banks of estuaries or in the mid to upper intertidal zone.[10−12] A collection of *S. oculus* (704 individuals) was made by hand at low tide, near Terrivonne Wharf in the Kariega River estuary, Eastern Cape, South Africa, in the spring of 2009. The *S. oculus* specimens were steeped in acetone, and the acetone extract was subjected to extensive chromatography to afford three new polypropionate metabolites, 6*Z*,8*E*-Δ^8-siphonarienfuranone (**1**), 6*E*,8*E*-Δ^8-siphonarienfuranone (**2**), and 6*E*,8*E*-3-hydroxy-4,6,8,10,12-pentamethylpentadeca-6,8-dien-5-one (**3**), and the known polypropionate siphonarienfuranone (**4**). The spectroscopic data obtained for **4** (0.04 mg/animal), previously isolated from *S. capensis*,[9] *S. grisea*,[13] and *S. pectinata*,[1] were consistent with literature data for this compound.[9,13] Although both *E*- and *Z*-siphonarienfuranone were isolated from the other three species only *E*-siphonarienfuranone was found in the extracts of *S. oculus* collected from the Kariega River estuary.

Polypropionate **1** (0.07 mg/animal) was isolated as a colorless oil, with a molecular formula established as $C_{20}H_{32}O_3$ from HREIMS data, which implied five degrees of unsaturation. The characteristic bands at 3339 and 1686 cm^{-1} in the IR spectrum of **1** indicated the presence of hydroxy and carbonyl functionalities, respectively. Inspection of the ^{13}C NMR and DEPT 135 spectra obtained for **1** revealed quaternary carbon signals at δ_C 102.0, 108.5, 184.8, and 202.8/202.9, suggesting the presence of a furanone moiety consistent with that found in **4**. This unsaturated ring system accounted for three of the five double-bond equivalents, and the remaining two degrees of unsaturation were accordingly assigned to two olefins in a side chain, which was confirmed by the presence of deshielded signals in the ^{13}C NMR spectra (δ_C 122.8/123.0, 130.7, 139.9/140.0, 141.5/141.8) and olefinic

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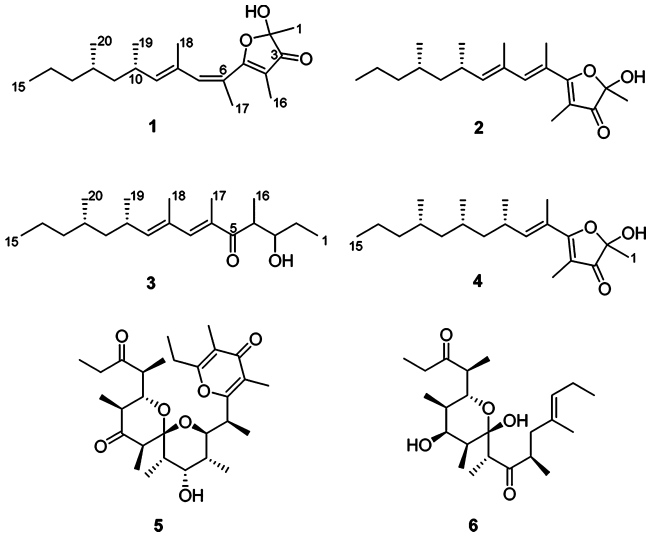

proton resonances [δ_H 5.20/5.23 (d, J = 9.8 Hz) and 6.26 (d, J = 1.2 Hz)] in the ^{1}H NMR spectrum. COSY correlations from the olefinic proton (δ_H 5.20/5.23, d, J = 9.8 Hz) to the methyl protons (δ_H 0.85, t, J = 7.2 Hz) indicated the presence of a contiguous seven-carbon chain with two methyl substituents, δ_H 0.79 (d, J = 6.20 Hz) and 0.87/0.85 (d, J = 6.5 Hz). The methylene/methyl envelope in the ^{1}H NMR spectrum of **1** also contained four other methyl proton signals, three of which were deshielded olefinic methyls at δ_H 1.52/1.53 (s), 1.69 (s), and 1.97/1.98 (d, J = 1.5 Hz). The duplication of the resonance for the olefinic methyl on the furanone ring (H_3-16, δ_H 1.52/1.53) in **1** arises from the presence of C-2 epimers of this compound. Further duplication of the proton and carbon resonances (with the exception of C-2) assigned to the furanone ring and side chain was also observed. The remaining methyl singlet (δ_H 1.55/1.56) was assigned to the methyl moiety attached to the hemiacetal carbon (δ_C 102) of the furanone ring.

The methyl group positions and that of the side chain were established through $^3J_{CH}$ gHMBC correlations from the methyl protons to the relevant surrounding carbon atoms (Figure 2 and Table 1). For example, the gHMBC correlation evident from H_3-1 (δ_H 1.55/1.56) to C-3 as well as those observed from H_3-16 (δ_H 1.52/1.53) to C-3 and C-5 confirmed the position of these methyl groups on the furanone ring. Further $^3J_{CH}$ gHMBC correlations from H_3-17 (δ_H 1.97/1.98) to C-5 and C-7 as well as those from H_3-18 (δ_H 1.69) to C-7 and C-9 finalized the structural assignment of the polypropionate side chain and confirmed its position at C-5 of the furanone ring.

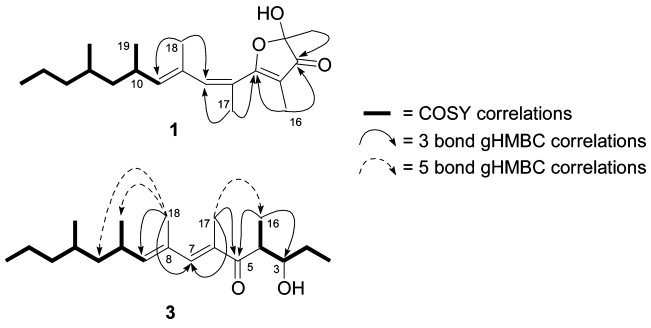

Figure 2. Key gHMBC and COSY correlations used to elucidate the structures of **1** and **3**.

The polypropionate **2** (0.01 mg/animal) was isolated as a colorless oil. The molecular formula of **2** was determined through HREIMS data as $C_{20}H_{32}O_3$, i.e., isomeric with **1**. The IR spectrum of **2** was consistent with that obtained for **1** and also confirmed the presence of hydroxy and carbonyl functionalities (ν_{max} 3429 and 1634 cm^{-1}, respectively). The quaternary carbon signals at δ_C 100.9, 106.8, 181.8, and 202.9 in the ^{13}C NMR and DEPT 135 spectra, as well as the methyl proton signals at δ_H 1.56 and 1.87 in the ^{1}H NMR, were also consistent with the 2-hydroxy-2,3-dihydro-2,4-dimethylfuran-3-one system of **1** and **4**. The presence of the olefinic carbon signals at δ_C 124.9, 130.3, 142.4, and 143.4/143.5 and deshielded olefinic methyl proton resonances at δ_H 1.88 and 2.12 in the ^{13}C and ^{1}H NMR spectra obtained for **2** accounted for the two remaining degrees of unsaturation. It was apparent from the fully assigned NMR data of **2** (Table 1) that this compound was a diastereomer of **1**, and NOESY NMR data were acquired for both **1** and **2**. There was a clear NOESY correlation between the methyl protons of H_3-17 (δ 2.12) and H_3-18 (δ 1.88) in the NOESY spectrum of **2**, while no correlation was observed between the analogous methyl protons at the same positions in **1**, thus implying that the methyl substituents at C-6 and C-8 are in relatively close proximity to each other in **2** and not **1**. A *Z*,*E* and an *E*,*E* configuration was accordingly proposed for **1** and **2** respectively. The chemical shift of the C-17 olefinic methyl (δ_C 21.8) provided further evidence in support of the *Z*-configuration of the Δ^6 olefin in **1** as opposed to the 6*E* configuration in **2**−**4** ($\underline{C}H_3$-17, δ_C ca. 13.2−15.4). The assignment of an *E* configuration to the Δ^8 olefins in **1**−**3** was similarly supported by the chemical shift of the olefinic methyl carbons attached to these functionalities.

Polypropionate **3** (0.03 mg/animal) was isolated as a yellow oil. The molecular formula of **3** ($C_{20}H_{36}O_2$) established from HREIMS data indicated three double-bond equivalents. The deshielded ^{13}C NMR chemical shift (δ_C 208.7) and the IR absorbance (ν_{max} 1639 cm^{-1}) suggested the presence of a ketone, which accounted for one of the three double-bond equivalents. The two remaining degrees of unsaturation were provided by four olefinic signals in the ^{13}C NMR spectrum of **3** (δ_C 134.7, 144.7, 130.8, and 144.5). A broad absorption at 3422 cm^{-1} in the IR spectrum as well as an oxymethine signal at δ_C 76.1 ppm in the ^{13}C NMR spectrum indicated the presence of a single hydroxy functionality. The upfield region of the ^{1}H NMR spectrum of **3** contained seven methyl proton signals, two of which were triplets (δ_H 0.87, t, J = 7.2 Hz and 0.98, t, J = 7.4 Hz), thus placing them at either end of the 15 carbon chain. The downfield chemical shift of two of the methyl resonances (δ_H 1.89, d, J = 1.0 Hz and δ_H 1.94, d, J = 1.0 Hz) suggested that they were olefinic methyl groups. The remaining unassigned doublet methyl proton signals at δ_H 0.85 (d, J = 6.5 Hz), 0.98 (d, J = 6.6 Hz), and 1.18 (d, J = 7.2 Hz) were positioned along the carbon chain using HSQC and COSY experiments. COSY correlations established the presence of two contiguous propionate chains, separated possibly by the quaternary carbons of the ketone and olefinic groups (Figure 2). The position of these two chains relative to the carbonyl and olefinic moieties was established through $^3J_{CH}$ and weak and unexpected, yet useful, $^5J_{CH}$ gHMBC correlations (Figure 2 and Table 1). $^3J_{CH}$ gHMBC correlations from the methyl protons at δ_H 1.18 to the oxymethine carbon (δ_C 76.1) and the carbonyl carbon (δ_C 208.7) positioned this methyl and its propionate chain adjacent to the ketone carbonyl (C-5). Further gHMBC correlations from the first olefinic methyl protons at δ_H 1.94 to

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Table 1. ^{1}H ($CDCl_3$, 600 MHz), ^{13}C ($CDCl_3$, 150 MHz), and HMBC NMR Data Obtained for 1, 2, and 3

	6*Z*,8*E*-Δ^8-siphonarienfuranone (**1**)			6*E*,8*E*-Δ^8-siphonarienfuranone (**2**)			6*E*,8*E*-3-hydroxy-4,6, 8,10,12-pentamethylpentadeca-6,8-dien-5-one (**3**)		
position	δ_C, type	δ_H (J in Hz)	HMBC[a]	δ_C, type	δ_H (J in Hz)	HMBC[a]	δ_C, type	δ_H (J in Hz)	HMBC[a]
1	22.2/22.3, CH_3	1.55/1.56, s	2, 3, 16	22.5, CH_3	1.56, s	2. 3	10.5, CH_3	0.98, t (7.4)	2, 3
2	102.0, C			100.9, C			28.2, CH_2	1.53, 1.44, m	1, 3, 4
3	202.8/202.9, C			202.9, C			76.1, CH	3.64, m	1, 2, 4, 5, 16
4	108.5, CH			106.8, CH			43.7, CH	3.35, quin (6.8)	2, 3, 5, 16
5	184.8, C			181.8, C			208.7, C		
6	122.8/123.0, C			124.9, C			134.4, C		
7	139.9/140.0, CH	6.26, d (1.2)	5, 9, 17, 18	142.4, CH	6.75, s	5, 9, 17, 18	144.7, CH	6.96, s	5, 6, 9, 17, 18, 19
8	130.7, C			130.3, C			130.8, C		
9	141.5/141.8, CH	5.20/5.23, d (9.8)	7, 11, 18	143.4/143.5, CH	5.36, d (9.7)	7, 11, 18, 19	144.5, CH	5.40, d (9.7)	7, 10, 11, 12, 18, 19
10	30.5, CH	2.49, m	8, 9, 11, 12, 19	30.8, CH	2.61, m	8	30.8, CH	2.61, m	8, 9, 11, 12, 19
11	45.2, CH_2	1.21, 1.05, m	9, 10, 12, 13, 19, 20	45.2, CH_2	1.32, dd (9.3, 4.0)	9, 10, 12, 13, 19, 20	45.1, CH_2	1.31, 1.13, m	8, 9, 13, 19
					1.13, dd (9.0, 4.5)				
12	30.6, CH	1.28, m	10, 11, 13	30.7, CH	1.38, m	14	30.7, CH	1.38, m	10, 14
13	40.1, CH_2	1.19, 1.06, m	11, 12, 15, 20	40.2, CH_2	1.24, 1.12, m	11, 12, 15, 20	40.1, CH_2	1.24, 1.11, m	11, 12, 15, 20
14	20.1, CH_2	1.29, 1.23, m	12, 13, 15	20.2, CH_2	1.31, m	12, 13, 15	20.2, CH_2	1.32, m	12
15	14.5/14.6, CH_3	0.85, t (7.2)	13, 14	14.5, CH_3	0.87, t (7.2)	13, 14	14.5, CH_3	0.87, t (7.2)	13, 14
16	6.3/6.4, CH_3	1.52/1.53, s	3, 4, 5, 6, 7, 17	8.0, CH_3	1.87, s	3, 4, 5	16.5, CH_3	1.18, d (7.2)	3, 4, 5
17	21.8/21.9, CH_3	1.98/1.97, d (1.5)	4, 5, 6, 7, 8, 16, 18	15.4, CH_3	2.12 br, s	5, 6, 7, 8	13.2, CH_3	1.94, d (1.0)	5, 6, 7, 8, 16
18	15.1/15.2, CH_3	1.69, s	6, 7, 8, 9, 10, 11, 19	16.8, CH_3	1.88 br, s	6, 7, 8,	16.7, CH_3	1.89, d (1.0)	7, 8, 9, 11, 19
19	21.0/21.2, CH_3	0.87/0.85, d (6.5)	9, 10, 11, 12	21.5, CH_3	0.98, d (6.7)	9, 10, 11	21.4, CH_3	0.98, d (6.6)	9, 10, 11
20	19.6/19.7, CH_3	0.79, d (6.2)	10. 11. 12. 13.	19.8, CH_3	0.85, d (6.4)	11, 12, 13	19.8, CH_3	0.85, d (6.5)	11, 12, 13

[a]HMBC correlations are from proton(s) stated to the indicated carbon.

C-5 and C-7 and from the second olefinic methyl protons at δ_H 1.89 to C-7 and C-9 placed them at C-17 and C-18, respectively, and established the conjugated arrangement of the ketone and the Δ^6 and Δ^8 olefins. This conjugated arrangement was confirmed from UV data (λ_{max} 276) and gHMBC correlations observed from the methyl protons on C-17 (δ_H 1.94) to C-16 and C-8 in addition to those from the C-18 methyl protons (δ_H 1.89) to C-11 and C-19.

The 10*S* and 12*S* absolute configuration in **4** is well-established and is congruent with the configuration assigned to these two stereogenic centers in all other similar marine polypropionates.[4] The co-occurrence and close structural similarities between **1**−**3** and **4** enabled us to extrapolate the same configuration to the analogous asymmetric carbons in **1**−**3** from biosynthetic arguments. The paucity of **3** that we had in hand prevented the assignment of the configuration at C-3 and C-4 in this compound.

The natural product status of cyclic marine polypropionate metabolites has been questioned in the past.[4,8,14] Paterson and Perkin[14] invoked a series of theoretical acyclic triketones as potential natural precursors for the cyclic polypropionates siphonarin B (**5**) and denticulatin A (**6**), respectively. Their observations of the facile interconversion of denticulatins A and B on silica gel suggested that in many cases cyclic marine polypropionates may represent thermodynamic and not enzymatic cyclization products of unstable acyclic precursors. They further hypothesized that cyclization occurs during the isolation of these compounds, and they remain convinced that many so-called cyclic polypropionate natural products are in fact isolation artifacts.[14]

Of interest to us therefore was the natural product status of **1**, **2**, and **4**. The standard natural product extraction method for marine mollusks (steeping in acetone for several days) does not establish whether the cyclic polypropionates, first, are present intact in the mucus immediately released when the *Siphonaria* are disturbed or, second, cyclize slowly after leaching out of the pedal glands into the acetone solution during extraction or later during chromatographic workup of the extract. Therefore, to investigate the polypropionate content in fresh mucus secretions, the lateral pedal glands of 36 *S. oculus* limpets were

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aggravated and the cloudy white mucus that exuded from the glands was collected in a series of capillary tubes (Figure 1). The mucus-containing capillary tubes were placed in $CHCl_3$, and the $CHCl_3$ extract was concentrated under reduced pressure. The ^1H NMR spectrum ($CDCl_3$, 600 MHz) of the resultant mucus extract (10.4 mg) was compared with the ^1H spectra obtained for each of the pure metabolites isolated from the bulk, acetone extraction of numerous *S. oculus* species (Figure 3). While the upfield region (δ_H 0.50−2.20) of the

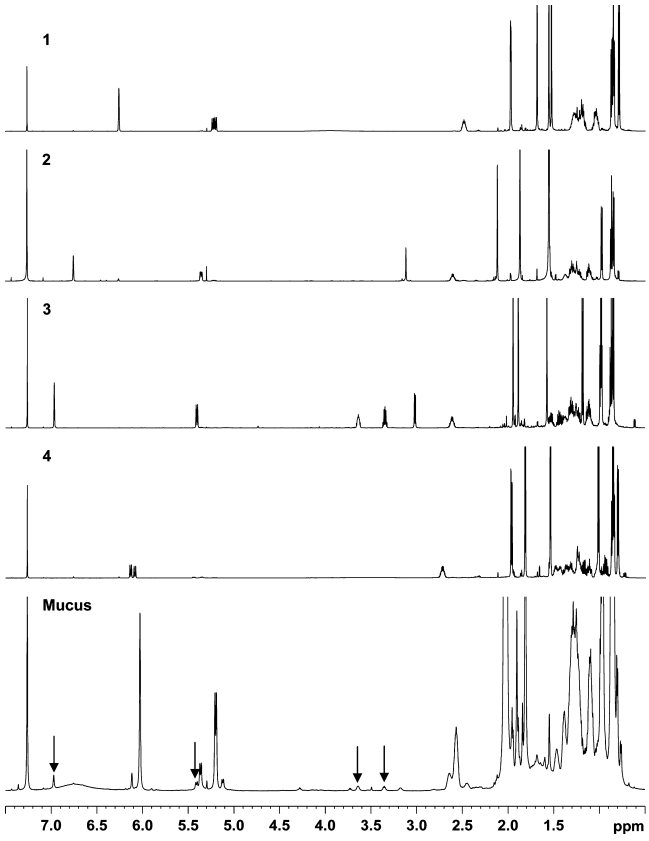


Figure 3. ^1H NMR spectra ($CDCl_3$, 600 MHz) of the isolated metabolites **1**−**4** and the mucus extract. Arrows highlight common deshielded resonances from **3**.

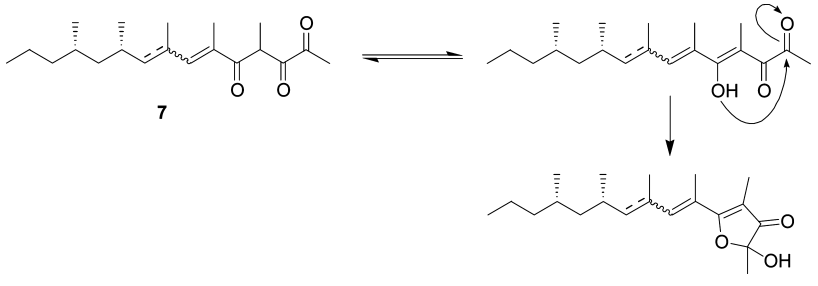
^1H NMR spectrum acquired from the mucus extract was complicated by numerous overlapping signals, the downfield region (δ_H 2.30 to 7.20) was greatly simplified, enabling confirmation of the presence of unsaturated polypropionate metabolites (Figure 3). From the relative signal intensities observed in the ^1H NMR spectrum of the mucus extract it would appear that the polypropionates are only minor constituents of the mucus exudate. Interestingly, the characteristic olefinic downfield doublets of the three compounds containing a furanone ring (**1**, **2**, and **4**) appear to be missing in the mucus extract, while those corresponding to either the linear polypropionate (**3**) or possibly a closely related analogue are evident. This observation might suggest that the furanone ring-containing compounds may be artifacts, arising from cyclization of a putative tautomeric acyclic triketone precursor (**7**) during chromatographic workup of the extract (Scheme 1).

We were also interested in exploring the structure and acidity within the lateral pedal glands in *S. oculus*. Sections of the lateral pedal glands of several *S. oculus* specimens were consequently prepared, stained, and examined with light microscopy (Figure 1). Toluidine blue is a metachromatic dye commonly used to stain mast cells, cartilage, and certain acid mucins and changes in color (red-purple) when exposed to different chromotropic tissue elements.[15] *S. oculus* sections stained with this dye clearly showed the complexity of the gland by revealing a number of different cell types incorporated within the gland (Figure 1). Alcian blue is a phthalocyanin dye primarily used for staining blue any acidic polysaccharides, acidic metabolites, and acidic cell components, while neutral areas within the cell are stained magenta.[15] The blue-stained areas in the center of the *S. oculus* pedal gland suggests that the contents of the lumen and the cells nearest to the lumen are acidic. Aldehyde fuchsin, an aminotriarylmethane dye activated by carboxylated groups to produce a deep purple color,[15] further supported the results obtained from the alcian blue sections, indicating the presence of acidic compounds in the cells surrounding the lumen. Interestingly, the acidity of the interior of the *S. oculus* lateral pedal gland does not appear to induce cyclization of putative acyclic precursors leading to **1**, **2**, and **4**.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 141 polarimeter at the sodium-D line (589 nm). Following standard protocol, the concentration of solutions used to determine optical rotations is expressed in g/100 mL. Ultraviolet spectra were recorded on a Shimadzu UV−vis 2550 spectrometer. Infrared spectra were recorded on a Perkin-Elmer Spectrum 2000 FT-IR spectrometer and Digilab FTS 3100 Excalibur HE Series with compounds as films (neat) on NaCl discs. NMR spectra were acquired using standard pulse sequences on a Bruker Avance 600 MHz Avance II spectrometer. Chemical shifts are reported in ppm and referenced to residual solvent resonances ($CDCl_3$ δ_H 7.26, δ_C 77.0). Coupling constants are reported directly from the NMR spectra, and corresponding coupling constants have not been matched. Mass spectrometry was performed on a Waters API Q-TOF Ultima instrument using electron-spray ionization in the positive ion

Scheme 1. Proposed Formation of 1, 2, and 4 from Putative Triketone Precursor 7



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mode (ESI+) at the University of Stellenbosch Central Analytical Facility. HP-20 beads used for chromatography were manufactured by Diaion and supplied by Supelco. Normal-phase DIOL semipreparative HPLC separations were performed on a Machery-Nagel VP 250/10 Nucleosil 100-7 OH column using an Agilent 1100 Series quad pump and an Agilent 1100 diode array detector. Reversed-phase semipreparative HPLC separations were performed on a Phenomenex Onyx Monolithic Semi-PREP C-18 column using an Agilent 1100 Series quad pump and an Agilent 1100 diode array detector.

Animal Material and Isolation Procedure. In August 2009, a collection of 704 specimens (303.3 g) of *Siphonaria oculus* (Krauss 1848) was made near Terrivonne Wharf, 3 km up the Kariega River estuary. A voucher specimen of the limpet (CLB2009-001) is retained in the Rhodes University marine invertebrate collection. The limpets were steeped in Me_2CO, and the extract was cyclic loaded onto HP-20 beads until a concentration of 12.5% Me_2CO/H_2O was achieved. The HP-20 resin was eluted with aqueous Me_2CO mixtures (20%, 40%, 60%, 80% Me_2CO/H_2O and 100% Me_2CO) to afford five fractions. The 80% aqueous Me_2CO fraction was purified further with normal-phase HPLC (20% EtOAc/hexane) on a DIOL column, affording seven fractions. Fraction 6 was subjected to reversed-phase HPLC (80% MeOH/H_2O) on a C_{18} column to afford polypropionates **1** (0.07 mg/animal) and **4** (0.04 mg/animal). Fraction 7 was purified further using reversed-phase HPLC (90% MeOH/H_2O), followed by a series of normal-phase HPLC purifications (100%, 20%, 10% EtOAc/hexane) on a DIOL column to afford **2** (0.01 mg/animal). Polypropionate **3** (0.03 mg/animal) was isolated from fraction 4 after normal-phase HPLC (10% EtOAc/hexane) on a DIOL column.

*6Z,8E-Δ^8-Siphonarienfuranone (**1**):* colorless oil; $[\alpha]^{20}_D$ −38 (*c* 0.97, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 232 (3.81), 305 (3.82) nm; IR (film) ν_{max} 3339, 3019, 2959, 2927, 2871, 1686, 1590, 1216 cm^{-1}; ^1H and ^{13}C NMR data see Table 1; EIMS *m*/*z* 320 [M^+] (16), 277 (10), 207 (100), 189 (26), 165 (40), 137 (94); HREIMS *m*/*z* 320.2350 [M^+] (calcd for $C_{20}H_{32}O_3$, 320.2351).

*6E,8E-Δ^8-Siphonarienfuranone (**2**):* colorless oil; $[\alpha]^{19}_D$ −160 (*c* 0.08, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 209 (sh) (3.60), 226 (3.60), 305 (3.57) nm; IR (film) ν_{max} 3429, 2957, 2845, 1634, 1459, 1381, 1018 cm^{-1}; ^1H and ^{13}C NMR data see Table 1; EIMS *m*/*z* 320 [M^+] (10), 277 (7), 207 (100), 189 (14), 163 (32), 137 (88); HREIMS *m*/*z* 320.2350 [M^+] (calcd for $C_{20}H_{32}O_3$, 320.2351).

*6E,8E-3-Hydroxy-4,6,8,10,12-pentamethylpentadeca-6,8-dien-5-one (**3**):* yellow oil; $[\alpha]^{19}_D$ +61 (*c* 0.35, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 209 (3.40), 276 (3.03) nm; IR (film) ν_{max} 3422, 2957, 2927, 1639, 1451, 1381, 1206 cm^{-1}; ^1H and ^{13}C NMR data see Table 1; HREIMS *m*/*z* 308.2860 [M^+] (calcd for $C_{20}H_{36}O_2$, 308.2715).

*Siphonarienfuranone (**4**) (refs 9, 13):* colorless oil; $[\alpha]^{20}_D$ +105 (*c* 0.53, $CHCl_3$), lit.[9,13] +102, +54; IR, NMR, and MS data consistent with published data;[9,13] HREIMS *m*/*z* 323.2572 [$(M + H)^+$] (calcd for $C_{20}H_{35}O_3$, 323.2586).

Microscopy. In the laboratory tissues from the lateral regions of the head and foot of five individuals were dissected, fixed, and processed for light microscopy (histology and histochemistry). Animals were not anaesthetized prior to fixation. To prepare tissues for staining in alcian blue (pH 1.5 and 2.4) and aldehyde fuchsin, some tissue was fixed for at least 24 h in aqueous Bouin's solution, dehydrated, and embedded in Paraplast via xylene. Sections 5−7 μm thick were cut on a Leica Rm 2035 rotary microtome. Staining protocols followed those given in Humason[16] and Bancroft and Gamble.[17] Small pieces of tissue to be stained in toluidine blue were fixed in 2.5% glutaraldehyde in filtered seawater (4 °C) for 12 h. Tissues were then rinsed in 0.2 M sodium cacodylate buffer (pH 7.2) followed by secondary fixation in 1% osmium tetroxide in 0.2 M sodium cacodylate buffer for 90 min. After rinsing in 0.2 M sodium cacodylate buffer, tissues were dehydrated through a graded EtOH series (30−100%) and embedded in an Araldite-Taab 812 resin mixture[18] via propylene oxide. Semithin sections of approximately 1 μm in thickness were cut with a diamond knife from the polymerized blocks using an RMC MT-7 ultramicrotome and then stained for 20 s in 1% toluidine blue.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Tel: +27466037030. Fax: +27466225109. E-mail: m.davies-coleman@ru.ac.za.

Notes

The authors declare no competing financial interest.

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■ DEDICATION

Dedicated to Dr. Gordon M. Cragg, formerly Chief, Natural Products Branch, National Cancer Institute, Frederick, Maryland, for his pioneering work on the development of natural product anticancer agents.

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