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Novel Polypropionates from the South African Marine Mollusc *Siphonaria capensis*

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Abstract: The endemic South African pulmonate mollusc *Siphonaria capensis* yielded C-2 epimeric mixtures of the known polypropionate metabolites siphonarienfuranone (**1**) and its Z-isomer (**2**), and three new polypropionates, capensinone (**3**), capensifuranone (**4**) and (2E, 4S, 6S, 8S)-2,4,6,8-tetramethyl-2-undecenoic acid (**5**).

Molluscs of the marine pulmonate genus *Siphonaria* are air-breathing intertidal herbivores, often referred to as false limpets. During high tide, siphonariids remain firmly fixed to depressions or "home-scars" in the rock surface. As the tide recedes these molluscs leave their home-scars to feed on algae and micro-organisms thus exposing themselves to predation by terrestrial predators, in addition to the aquatic predators *e.g.* tidepool fish encountered when they are submerged. Shortly after being disturbed by a potential predator, siphonariids produce a white mucus, containing polypropionate metabolites, from lateral pedal glands.[1] A feature of *Siphonaria* polypropionate compounds (derived from multiple condensations of propionate units) is their frequent cyclization to yield furanone, pyrone and hemi-acetal functionalities.[2] Surprisingly, the role of these compounds (or their hypothetical acyclic precursors) in the chemical ecology of *Siphonaria* species, is often poorly understood.[2] In continuation of our search for new bioactive compounds from southern African marine molluscs[3-5] we have examined specimens of *S. capensis* from near the Bushman's River mouth on the south-east coast of South Africa. The endemic *S. capensis*, is the most common of the nine known South African *Siphonaria* species.[6]

Specimens of *S. capensis* were steeped in acetone, the acetone decanted and the molluscs extracted repeatedly with further acetone. Concentration under reduced pressure of the combined acetone extracts, followed by partitioning between ethyl acetate and water, gave a crude ethyl acetate partition fraction which was chromatographed on silica (1:2 EtOAc/hexane). Further normal phase (1:4 EtOAc/hexane) and reverse phase (9:1 MeOH/H_2O) HPLC of a portion of one of the chromatography fractions, adjudged by NMR spectroscopy to contain polypropionate metabolites, yielded C-2 epimeric mixtures of siphonarienfuranone (**1**) and the Z-isomer of this compound (**2**), capensinone (**3**), capensifuranone (**4**) and (2E, 4S, 6S, 8S)-2,4,6,8-tetramethyl-2-undecenoic acid (**5**).

The two geometric isomers, E and Z siphonarienfuranone (**1** and **2**) have been isolated previously, also as C-2 epimeric mixtures, from *S. grisea,*[7] and *S. pectinata.*[8] The NMR data for the epimeric mixtures of **1** and **2**, isolated from *S. capensis*, including the characteristic duplication of the C-2 and C-5 ^{13}C NMR signals (δ101.0/ 101.1 and 181.8/ 182.0 respectively) were in accordance with published values.[7] The slow isomerization

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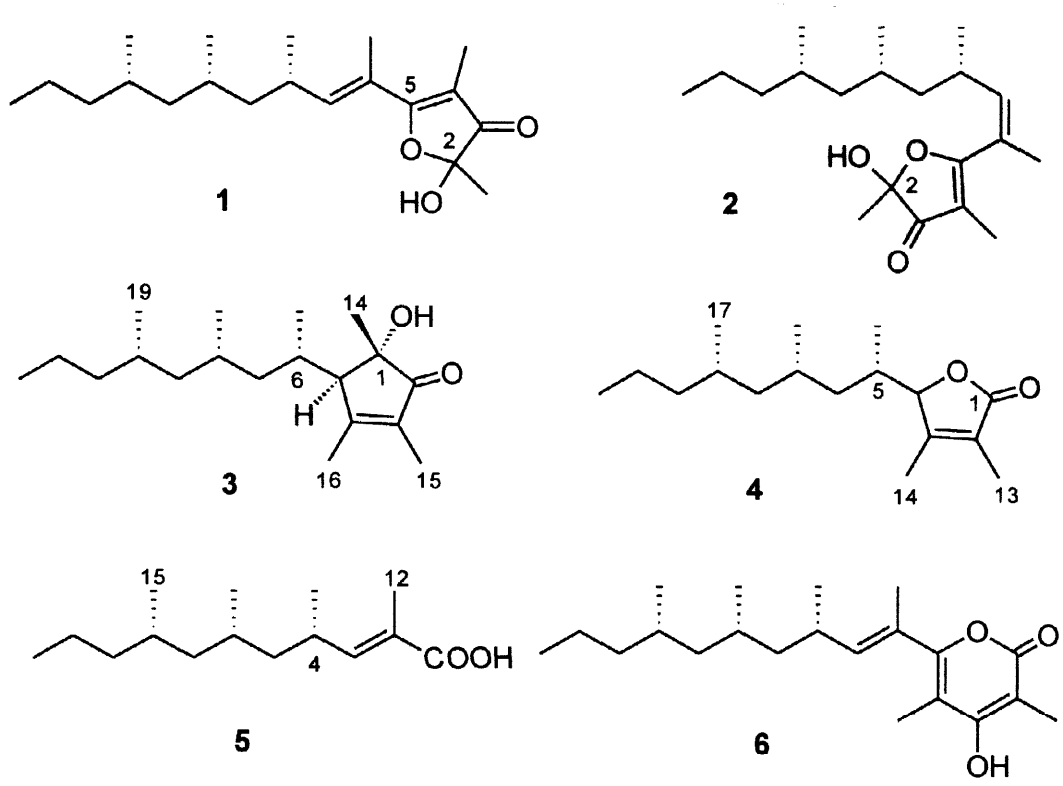

of **1** to **2** on standing was also observed from ^{1}H NMR analysis of **1** over several days. Although the significant difference between the optical rotation of the mixture of **1** ($[\alpha]_D$ + 54, *c* 0.18, $CHCl_3$) from *S. capensis* and that reported by Norte *et al.*[7] for the same mixture from *S. grisea* ($[\alpha]_D$ +102, *c* 0.14, $CHCl_3$) probably reflects this isomerization, a chemical investigation of the side chain stereochemistry of this compound was nonetheless deemed necessary. Oxidative cleavage of **1** with ruthenium tetroxide[9] gave (2S, 4S, 6S)-2,4,6 trimethylnonanoic acid ($[\alpha]_D$ + 15, *c* 0.50, $CHCl_3$; lit.[7] +15, *c* 0.6, $CHCl_3$) as the major product, thus confirming the expected absolute stereochemistry of the three chiral centres in the side chain of **1** and hence **2**. Fortuitously, a sample of the ubiquitous polypropionate, pectinatone[7,8,10,11] (**6**, $[\alpha]_D$ +66, *c* 0.17, $CHCl_3$; lit[7] +64, *c* 0.11, $CHCl_3$), was at hand from a previous investigation of the polypropionate constituents of the South African siphonariid *S. concinna*.[12] Ruthenium tetroxide oxidation of **6** from *S. concinna* thus not only corroborated the consistency of the oxidative method employed but also provided a suitable NMR standard of (2S, 4S, 6S)-2,4,6 trimethylnonanoic acid ($[\alpha]_D$ + 14, *c* 0.54, $CHCl_3$) for comparison with the oxidative degradation products of **1** and **5**.

A minor polypropionate metabolite in the *S. capensis* extract, isolated as a yellow oil ($[\alpha]_D$ -65, *c* 0.42, $CHCl_3$), was the new cyclopentenone, capensinone. A molecular formula of $C_{19}H_{34}O_2$ for **3** was deduced from the NMR data and confirmed by the HREI mass spectrum (*m/z* 294.2546, Δmmu -13). Of the three double bond equivalents required by the molecular formula, two could be assigned to an αβ-unsaturated ketone (δ_C 210, 133, 171 ppm and IR υ_{max} 1700, 1630 cm^{-1}). The remaining degree of unsaturation, unaccounted for from the deshielded ^{1}H and ^{13}C NMR resonances, implied that capensinone also possessed a single ring.

Table 1. ^{13}C **(100 MHz, $CDCl_3$) and** ^{1}H **(400 MHz, $CDCl_3$) NMR data for compounds 3 and 4.**

Compound	3		4	
Carbon	δ_C ppm(Mult)	δ_H ppm (Mult, J/Hz)	δ_C ppm(Mult)	δ_H ppm (Mult, J/Hz)
1	77.3 (s)	-	174.8 (s)	-
2	209.7 (s)	-	124.6 (s)	-
3	133.4 (s)	-	158.0 (s)	-
4	170.8 (s)	-	87.8 (d)	4.68 (brs)
5	58.0 (d)	2.73 (brs)	32.1 (d)	2.03 (m)
6	29.8 (d)	2.22 (m)	36.6 (t)	0.90 (m)
7	41.9 (t)	1.45 (m), 1.10 (m)	29.8 (d)	1.46 (m)
8	28.1 (d)	1.70 (m)	44.5 (t)	1.16 (m), 0.78 (m)
9	44.9 (t)	1.25 (m), 0.90 (m)	27.7 (d)	1.47 (m)
10	29.7 (d)	1.52 (m)	38.4 (t)	1.28 (m), 0.96 (m)
11	38.7 (t)	1.30 (m), 1.02 (m)	19.8 (t)	1.33 (m), 1.22 (m)
12	19.9 (t)	1.35 (m), 1.25 (m)	14.4 (q)	0.87 (m)
13	14.4 (q)	0.90 (m)	8.4 (q)	1.82 (s)
14	22.2 (q)	1.32 (s)	12.4 (q)	1.93 (s)
15	8.2 (q)	1.75 (s)	17.5 (q)	1.10 (d, 7)
16	16.0 (q)	2.04 (s)	21.2 (q)	0.83 (d, 7)
17	18.2 (q)	0.97 (d, 7)	20.7 (q)	0.83 (d, 7)
18	20.6 (q)	0.95 (d, 7)		
19	20.8 (q)	0.87 (d, 7)		

The ^{1}H NMR spectrum of **3**, which revealed seven methyl proton signals: three doublets (δ 0.87, 0.95, and 0.97 ppm), a multiplet (δ 0.90 ppm) and three singlets (δ 1.32, 1.75 and 2.04 ppm), immediately suggested a polypropionate skeleton for this compound. The latter two deshielded methyl singlets were assigned to two olefinic methyl groups, while the chemical shift of the third methyl singlet was consistent with a methyl moiety attached to an hydroxylated quaternary carbon (δ_C 77 ppm). A broad IR absorbance at 3450 cm^{-1} confirmed the presence of an alcohol functionality in **3**. A combination of COSY-90, DEPT-135 and HMQC NMR experiments and recourse to the ^{1}H and ^{13}C data for **1** and **2**, were used to interpret the overlapping ^{1}H NMR resonances in the methylene envelope (δ 0.8 - 1.5 ppm) and delineate the structure of the characteristic, polypropionate derived, 1,3,5-trimethyloctyl side chain.

The structure of the saturated side chain of **3** and the single ring implied by the molecular formula, required the olefin and its associated methyl groups, the carbonyl group and the tertiary alcohol moiety to constitute a highly substituted cyclopentenone ring. Definitive two and three bond HMBC correlations from H-5 (δ 2.73 ppm) to C-1, C-4 and C-17 and from 3H-15 to C-2, C-3 and C-4 unequivocally established the

substitution pattern around this ring. 1D-NOE difference experiments were used to explore the relative stereochemistry at C-1 and C-5. The absence of an NOE enhancement of the C-14 methyl protons on irradiation of H-5 and *vice versa* tentatively suggested the relative stereochemistry shown. The all (S)-absolute configuration, established for the three chiral centres in the side chain of **1** and **2**, is extrapolated to the analogous asymmetric carbon atoms in **3** from the assumption that these compounds arise through a common biosynthetic pathway.[2] The ^{1}H and ^{13}C NMR data for **3** is presented in Table 1.

HREIMS data provided the molecular formula ($C_{17}H_{30}O_2$, *m/z* 266.2236, Δmmu -10) of the second new minor metabolite capensifuranone ($[\alpha]_D$ -15, *c* 0.29, $CHCl_3$). The ^{1}H NMR spectra of **3** and **4** were superficially similar, however, closer examination of the latter spectrum revealed that firstly, the deshielded proton resonance (δ 2.73 ppm) in the former spectrum was shifted further downfield (δ 4.68 ppm) and that secondly, one methyl singlet (δ 1.32 ppm) was missing. The ^{13}C NMR and DEPT-135 spectra of **4** proved more informative, with the chemical shifts of three deshielded, quaternary resonances at δ 174, 158 and 124 ppm consistent with those expected for a di-substituted, αβ-unsaturated lactone functionality. In the absence of IR evidence to support the presence of an alcohol group in **4**, the single oxymethine ^{13}C resonance at δ 88 ppm was also assigned to the lactone ring. Definitive HMBC correlations from the 3H-14 olefinic methyl protons to the oxymethine carbon (C-4), and the vinylic carbons (C-3 and C-2) established the presence of a tri-substituted, αβ-unsaturated-γ-lactone ring system in **4** which consequently fulfilled the double bond equivalent requirements of the molecular formula. Standard 2D NMR also established the structure of the 1,3,5-trimethyloctyl side chain in **4** and contributed to the assignment of the ^{1}H and ^{13}C NMR chemical shifts (Table 1). An all (S)-stereochemistry for the acyclic chiral centres in **4** was again proposed from biosynthetic arguments. The configuration at C-4 remains unassigned.

The molecular formula of compound **5** ($C_{15}H_{28}O_2$), established from HREIMS data(*m/z* 240.2092, Δmmu +3) required two degrees of unsaturation. The deshielded carbonyl (δ 173 ppm) and vinylic carbon (δ 151 and 125 ppm) signals in the ^{13}C-NMR spectrum for **5** together with the hydroxyl IR absorbance (υ_{max} 3000 cm^{-1}) supported the presence of an αβ-unsaturated carboxylic acid functionality and hence required this compound to be acyclic. Similarities between the ^{13}C and ^{1}H NMR data of **5** and **1**, together with standard 2D-NMR experiments, clinched the polypropionate structure of the molecule with the ^{13}C chemical shift of the single olefinic methyl (δ 12.1 ppm) defining the E-configuration of the vinylic moiety (a Z-configuration requires an olefinic methyl chemical shift of δ_C 20.9 ppm).[6] The (2E, 4S, 6S, 8S)-2,4,6,8-tetramethyl-2-undecenoic acid structure of **5** was conclusively confirmed by oxidative degradation to (2S, 4S, 6S)-2,4,6 trimethylnonanoic acid ($[\alpha]_D$ + 19, *c* 0.25, $CHCl_3$). The all (S)-configuration in **5** further vindicates the tentative assignment of an (S) absolute stereochemistry to each of the three acyclic chiral centres in **3** and **4**.

Polypropionate metabolites with five-membered rings are rare in the marine environment. The structure of five membered rings in polypropionates isolated thus far from marine molluscs has been limited to furanones,[2] and capensinone is therefore the first example of a marine polypropionate metabolite containing a cyclopentenone moiety. The vicinal arrangement of the two olefinic methyl groups in both **3** and **4** is also most unusual for polypropionate derived metabolites and the biosynthesis of these compounds is unclear. The biosynthetic incorporation of acetate units into a predominantly propionate derived compound has been established from a labelling study of siphonarin A biosynthesis in *S. zelandica*[13] and recently proposed for the biosynthesis of cyercenes 1 - 4 and cyercene B from the sacoglossan mollusc *Cyerce cyrstallina*.[2] It is possible that similar

mixed biosynthetic pathways may give rise to **3** and **4**. The biosynthesis of **5** from five propionate units is unambiguous.

Experimental Section

The ^1H (400MHz) and ^{13}C (100MHz) NMR spectra were recorded on a Bruker AMX400 spectrometer. Optical rotations were measured on a Perkin-Elmer 141 polarimeter. Low resolution mass spectra were recorded on a Hewlett-Packard 5988A spectrometer and high resolution spectra were obtained by Dr P. Boshoff of the Mass Spectrometry Unit at the Cape Technikon, Cape Town. High-performance liquid chromatographic separations were performed on a Whatman Magnum 9 Partisil and Phenomenex C-18 columns.

Collection and Extraction of *Siphonaria capensis*: 558 specimens of *S.capensis* were collected from the intertidal zone at the Bushman's River mouth, South Africa in the summer of 1992 and stored in acetone until work up. The extract was decanted and the specimens were re-extracted with acetone. The acetone extracts were pooled, concentrated and partitioned between EtOAc and water. The polypropionate compounds obtained from the concentrated EtOAc partition layer by silica chromatography (1:2 EtOAc/hexane), normal phase HPLC (1:4 EtOAc/hexane) and C-18 reverse phase HPLC (9:1 MeOH/H_2O) were C-2 epimeric mixtures of siphonarienfuranone (0.07mg/animal) and the Z-isomer of this compound (0.04 mg/animal), capensinone (0.03 mg/animal), capensifuranone (0.02 mg/animal) and (2E, 4S, 6S, 8S)-2,4,6,8-tetramethyl-2-undecenoic acid (0.02 mg/animal). The ^1H and ^{13}C NMR and IR data for compounds **1** and **2** were consistent with published values.[7]

Capensinone (3): oil; $[\alpha]_D^{21}$ = - 65 (*c* 0.42, $CHCl_3$); IR (film) 3450, 2960, 2920, 2840, 1700, 1630, 1450, 1370 cm^{-1}; ^1H and ^{13}C NMR data see Table 1; EIMS (70eV), *m/z* (int, %), 139 (100), 123 (10), 122 (31), 111 (15), 71 (8), 55 (13), 43 (34); CIMS (CH_4, 1.8 Torr) 295 (100), 294 (6), 277 (80); HREIMS obsd. *m/z* 294.2546, $C_{19}H_{34}O_2$ (M^+) requires 294.2559.

Capensifuranone (4): oil; $[\alpha]_D^{21}$ = - 15.1 (*c* 0.29, $CHCl_3$); IR (film) 3440, 2960, 2920, 2840, 1720, 1450, 1370 1250 cm^{-1}; ^1H and ^{13}C NMR data see Table 1; EIMS (70eV), *m/z* (int, %), 139 (24), 113 (14), 112 (100), 111 (16), 99 (11), 85 (28), 71 (23), 57 (28); HREIMS obsd. *m/z* 266.2236, $C_{17}H_{30}O_2$ (M^+) requires 266.2246.

(2E, 4S, 6S, 8S)-2,4,6,8-Tetramethyl-2-undecenoic acid (5): oil; $[\alpha]_D^{21}$ = +32 (*c* 0.19, $CHCl_3$); IR (film) 3000, 2980, 2900, 1780, 1700, 1650, 1460, 1425, 1380, 1280 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.65 (m, 1H, H-3), 2.63 (m, 1H, H-4), 1.85 (s, 3H,3H-12), 1.48 (m, 1H, H-8), 1.38 (m, 1H, H-6), 1.36 (m, 1H, H-5a), 1.32 (m, 1H, H-10a), 1.24 (m, 2H, H-9a, H-10b), 1.15 (m, 1H, H-7a), 1.10 (m, 1H, H-5b), 1.02 (m, 1H, H-9b), 1.00 (d, 3H, $J_{4,13}$ = 7Hz, 3H-13), 0.90 (m, 1H, H-7b), 0.88 (m, 3H, 3H-11), 0.83 (d, 3H, $J_{6,14}$ = 7Hz, 3H-14), 0.82 (d, 3H, $J_{8,15}$ = 7Hz, 3H-15); ^{13}C NMR ($CDCl_3$) δ 173.3 (s, C-1), 151.0 (d, C-3), 125.4 (s, C-2), 45.6 (t, C-7), 44.3 (t, C-5), 39.3 (t, C-9), 31.1 (d, C-4), 29.6 (d, C-8), 28.2 (d, C-6), 20.5 (q, C-13), 20.4 (q, C-14), 20.0 (t, C-10), 19.9 (q, C-15), 14.4 (q, C-11), 12.1 (q, C-12); EIMS (70eV), *m/z* (int, %), 167.0 (40), 150.0 (12), 148.9 (100), 71.2 (14), 70.2 (11), 57.2 (19), 55(9); CIMS (CH_4, 1.8 Torr) 269 (8), 241 (100), 223 (17); HREIMS obsd. *m/z* 240.2092, $C_{15}H_{28}O_2$(M^+) requires 240.2089.

Oxidative degradation with ruthenium tetroxide: The following method is representative. Compound **5** (6.7 mg, 0.028 mmol) was dissolved in CCl_4 (0.5 ml) and added to a solution of CH_3CN (0.5 ml), H_2O (0.75 ml), periodic acid (30 mg, 0.132 mmol) and a catalytic amount of $RuCl_3$. $3H_2O$. The reaction mixture was stirred vigorously (4.5 h) at room temperature, concentrated, and partitioned between H_2O (5 ml) and CH_2Cl_2 (3 x 5 ml). The combined CH_2Cl_2 partition fractions were dried, concentrated and subjected to normal phase HPLC (EtOAc/Hexane 3:7) to give (2S, 4S, 6S)-2,4,6 trimethylnonanoic acid as an oil; 2.5 mg (45% yield); $[\alpha]_D$ = +19 (*c* 0.25, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 2.58 (m, 1H), 1.74 (m, 1H), 1.53 (m, 2H), 1.18 (d, 3H, J = 7Hz), 0.88 (m, 3H), 0.88 (m, 3H), 0.82 (d, 3H, J = 7Hz); ^{13}C NMR ($CDCl_3$) δ 181.9 (s), 45.3 (t), 41.2 (t), 39.2 (t), 37.1 (d), 29.6 (d), 28.2 (d), 20.3 (q), 20.0 (q), 19.9 (t), 18.0 (q), 14.4 (q).

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