A New Furanosesterterpene from the South African Nudibranch *Hypselodoris capensis* and a Dictyoceratida Sponge

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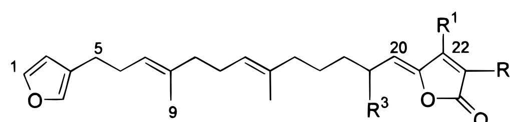
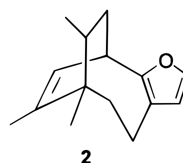
The known cytotoxic, linear *β*-substituted sesterterpenes (18*R*)-variabilin (**1**) and 22-deoxyvariabilin (**4**) and a new variant, 22-deoxy-23-hydroxymethylvariabilin (**5**), were isolated from the endemic South African nudibranch *Hypselodoris capensis* together with the known ichthyotoxic sesquiterpenes nakafuran-8 (**2**) and -9 (**3**). One of *H. capensis'* dietary sponges, a *Fasciospongia* sp. collected simultaneously with the nudibranchs, yielded sesterterpenes **1**, **4**, and **5** and the ubiquitous prenylated furan derivative, furospinosulin-1 (**7**).

The endemic South African nudibranch *Hypselodoris capensis* is a colorful member of the family Chromodorididae, a group of soft-bodied opisthobranch mollusks whose striking coloration implies a chemical defense strategy.[1] Dorid nudibranchs are specialist feeders that have evolved the ability to selectively take up and store, in their mantle tissue, bioactive metabolites from sponges rarely fed upon by other organisms. Therefore, in continuation of our search for new bioactive metabolites from Southern African nudibranchs we have examined the predator−prey relationship between the nudibranch *H. capensis* and the *Fasciospongia* sponge on which it was found during a recent scuba collection of invertebrates from the Tsitsikamma Marine Reserve on the southeastern coast of South Africa. *H. capensis* yielded the previously reported (18*R*)-variabilin (**1**), nakafurans-8 (**2**) and -9 (**3**), and 22-deoxyvariabilin (**4**), together with a new variant, 22-deoxy-23-hydroxymethylvariabilin (**5**), while the *Fasciospongia* sponge yielded furospinosulin-1 (**7**) in addition to compounds **1**, **4**, and **5**.

The EtOAc partition fraction of an Me_2CO extract of 16 specimens of *H. capensis* was chromatographed on Si gel to give six crude fractions. The known furanosesquiterpene compounds **2** and **3** were identified as the major constituents of the crude, nonpolar (hexane) chromatography fraction. The isolation of these two compounds concurs with the trend observed in *Hypselodoris* nudibranchs, which generally sequester furanosesquiterpene antifeedant metabolites from their dietary sponges.[2] The structure of **3** was fully assigned from coherent 1D and 2D NMR spectral data, while **2** was identified only from its ^{1}H NMR spectrum[3] and characteristic IR absorbances (2910, 1450, 1161 cm^{-1}). Unfortunately, an attempt to further purify **2** by reversed-phase HPLC resulted in the degradation of this unstable compound. Exhaustive HPLC of the remaining Si gel chromatography fractions yielded small amounts of *β*-substituted furans, as evidenced by characteristic signals (*δ* 7.32, 7.19, 6.26) in the ^{1}H NMR spectra of these HPLC fractions. At this point, hampered by a paucity of nudibranch material, and mindful of the notorious instability of *β*-substituted furans,[4] we decided to purify the *Fasciospongia* sponge extract, in the hope of isolating sufficient amounts of pure *β*-substituted furans for which acceptable spectral data could be obtained in as short a time as possible. Further support for this course of action was provided by a preliminary ^{1}H NMR examination of the sponge extract, which revealed *β*-substituted furan compounds similar to those evident in the *H. capensis* HPLC fractions.



1 R^1 = OH, R^2 = Me, R^3 = α - Me
4 R^1 = H, R^2 = R^3 = Me
5 R^1 = H, R^2 = CH_2OH, R^3 = Me
6 R^1 = H, R^2 = CH_2OAc, R^3 = Me



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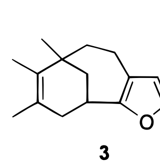

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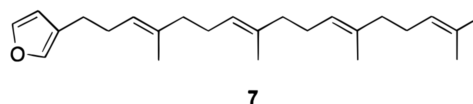

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An EtOAc partition fraction of the MeOH extract of the sponge was accordingly applied directly to a normal-phase HPLC column to give compounds **1**, **4**, **5**, and **7** in reasonable yield. Comparison of the ^{1}H NMR spectra of these four compounds found in the sponge with those of the linear sesterterpenes isolated from the nudibranchs showed that all except **7** were present in *H. capensis.* Variabilin (**1**) and furospinosulin-1 (**7**) were identified by comparing their NMR and IR spectral data with those reported previously.[5,6] The positive optical rotation obtained for **1** (+36°) is consistent with that reported by Martinez et al.[7] for an (*R*)-configuration at C-18 in this compound.

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Table 1. ^{13}C NMR Data for 22-Deoxyvariabilin[8] and Compounds **4** and **5**[a]

carbon	compound		
	"4"[8]	**4**	**5**
1	142.5	142.5 d	142.5 d
2	111.1	111.1 d	111.1 d
3	124.9	125.0 s	125.0 s
4	138.8	138.8 d	138.8 d
5	25.0	25.0 t	25.0 t
6	28.4	28.5 t	28.5 t
7	123.7	123.7 d	123.8 d
8	135.8	135.8 s	135.8 s
9	15.8	16.0 q	16.0 q
10	39.5	39.5 t	39.5 t
11	26.5	26.6 t	26.6 t
12	124.3	124.4 d	124.5 d
13	134.8	134.8 s	134.8 s
14	16.0	15.8 q	15.8 q
15	39.7	39.7 t	39.7 t
16	25.7	25.7 t	25.7 t
17	36.4	36.7 t	36.6 t
18	31.3	31.3 d	31.6 d
19	20.8	20.7 q	20.6 q
20	120.6	120.5 d	123.2 d
21	128.9	147.5 s	147.5 s
22	137.9	137.8 d	137.9 d
23	125.3	128.9 s	131.8 s
24	170.1	171.2 s	172.8 s
25	10.5	10.5 q	56.9 t

[a] Values in ppm (mult.).

Comparison of the spectral data for compounds **4** and **5** with those of **1** showed that these compounds varied only in their substitution pattern around the tetronic acid moiety. Although the spectral data of compound **4** is generally consistent with that of 22-deoxyvariabilin,[8] a significant discrepancy was apparent on comparison of our ^{13}C data with the reported data for this compound (Table 1). Kernan et al.[8] assigned the ^{13}C signals at δ 128.9 to C-21 and δ 125.3 to C-23 for 22-deoxyvariabilin. The analogous C-21 and C-23 chemical shifts for variabilin are δ 143.0 and 99.1, respectively.[5] However, examination of our ^{13}C NMR spectra revealed no signal at δ 125.3, while a prominent resonance at δ 147.5, which showed two-bond HMBC correlations to H-20 (4.93) and H-22 (6.94), together with a very weak (4-bond) coupling to Me-25 (1.97), caused us to assign this signal to C-21. Because only the H-22 and Me-25 resonances showed correlations to the ^{13}C signal at δ 128.9, this signal was assigned to C-23. Our ^{1}H spectral data for compound **4** are identical to those reported for 22-deoxyvariabilin, while 1D NOE difference experiments showed reciprocal enhancement of the H-22 and H-20 resonances as expected for the structure given. IR spectral data were also in agreement with that reported by Kernan et al.,[8] the absence of a broad absorption centered around 3400 cm^{-1} being indicative of the deoxy compound. Interestingly, this appears to be the first report of the co-occurrence of variabilin and 22-deoxyvariabilin in the same organism. It was previously suggested that these two compounds, which vary only in the oxidation of their tetronic acid moieties, were characteristic of separate groups of sponge genera.[8] Although sesterterpene tetronic acids have been isolated from often misidentified sponges, later shown to be *Fasciospongia* species,[8,9] there has been no prior report of the occurrence of variabilin in this genus. The affinity of *Fasciospongia's* terpene chemistry with that of the *Ircinia/Psammocinia/Sarcotragus* group of sponges has been remarked upon previously.[9]

A molecular formula of $C_{25}H_{34}O_4$ for **5** was confirmed from HREIMS data, while a broad hydroxyl absorption band from 3660 to 3130 cm^{-1} in the IR spectrum of **5** supported the presence of an alcohol functionality in this compound. The only major differences between the ^{13}C NMR data of **4** and **5** were the absence of a methyl resonance at δ 10.5 and the presence of an additional methylene signal at δ 56.9 in the ^{13}C NMR spectrum of **5**, indicating a different substituent at C-23 on the tetronic acid moiety of this compound. A downfield shift of the C-23 signal from δ 128.9 for **4** to 131.8 for **5**[10] (Table 1), and a broad 3H singlet at δ 4.50 in the ^{1}H NMR spectrum, suggested a hydroxymethyl substituent at this position, with the hydroxyl proton signal overlapping that of the methylene protons. The presence of this hydroxymethyl moiety was confirmed by acetylation of **5** to give **6**, which resulted in a downfield shift of the methylene proton singlet from δ 4.50 to 4.88, and the appearance of an acetate methyl signal at δ 2.10 in the ^{1}H NMR spectrum of **6**. All other proton chemical shifts remained essentially unchanged. Hydroxymethyl substituents have previously been noted in difuranosesterterpene tetronic acids isolated from Australian sponges of the genera *Taonura*[8] and *Spongia*.[11]

β-Substituted furanosesterterpene tetronic acids have elicited much interest in the past 25 years due to their toxicity and their ubiquitous occurrence in marine Dictyoceratida sponges. Surprisingly, these compounds are uncommon in *Hypselodoris* nudibranchs, and, prior to this investigation, no linear furanosesterterpenes had been isolated from this genus.[2] Presumably the absence of **7** in the *H. capensis* extracts indicates selective sequestration of the *Fasciospongia* metabolites by this nudibranch. Although there is no previous evidence that *Hypselodoris* nudibranchs are capable of modifying sequestered sponge metabolites, it would be tempting to surmise that **7** is taken up by *H. capensis* and rearranged to give the nakafurans that were not found in the sponge. However, nakafurans-8 and -9, potent fish antifeedants, are known *Dysidea* sponge metabolites and have previously been reported to be sequestered by *Hypselodoris* and *Chromodoris* nudibranchs, which feed on *Dysidea* sponges.[2,3] The aposematic coloration of *Hypselodoris* species and the presence of mantle dermal formations for the storage of sequestered metabolites is thought to be crucial in allowing these nudibranchs to feed on a variety of sponges.[12] Therefore, although the nudibranchs used in this study were observed on the *Fasciospongia* sponge with which they were collected, it is possible that they had selectively sequestered nakafurans-8 and -9 from surrounding *Dysidea* sponges. We hope to return to our study site to identify further *H. capensis* dietary sponge species.

Experimental Section

General Experimental Procedures. The ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectra were recorded on a Bruker AMX400 spectrometer. Optical rotations were measured on a Perkin–Elmer 141 polarimeter. LRMS were recorded on a Hewlett–Packard 5988A spectrometer, and HRMS were obtained by Dr. P. Boshoff of the Mass Spectrometry Unit at the Cape

Technikon, Cape Town. HPLC separations were performed on a Whatman Magnum 9 Partisil column.

Animal Material. Sixteen specimens of the nudibranch *Hypselodoris capensis* were collected using scuba (−10 m) in the Tsitsikamma National Park (34°01′ S, 23°54′ E), a coastal reserve on the southeastern coast of South Africa, together with a portion of the *Fasciospongia* sponge on which they were found. The sponge is massive, horizontally extended and lobate, with the large oscula (3-mm diameter) along the raised ridges. Live coloration is a black-brown exterior with a lighter brown interior. It is compressible and spongy. The surface appears hispid when the fasciculated primary fibers penetrate the dermis. Primary fibers (65 μm in diameter) near the surface, contain foreign spicules, whereas the secondary fibers (30 μm in diameter) are most often clear. Specimens exude copious amounts of mucus soon after collection, and when dried, the skeleton is a reddish brown network of fibers. The sponge is an undescribed species of the genus *Fasciospongia* (order Dictyoceratida, family Thorectidae). A voucher specimen has been deposited at the South African Museum, Cape Town, South Africa (SAMA 24697).

Extraction and Isolation. The nudibranchs were stored in Me_2CO (100 mL) for one week, while the sponge (302 g wet mass) was steeped in MeOH over the same period. These initial extracts were partitioned between EtOAc and H_2O to yield 309 mg and 953 mg of crude extract, respectively. The nudibranch extract was chromatographed on a Si gel column eluted with a solvent gradient system of pure hexane, hexane−EtOAc 9:1, 8:2, 6:4, 1:1, and pure EtOAc. Six crude fractions were obtained by combining column fractions with similar TLC profiles. Normal-phase HPLC (hexane) was used to purify the least polar of these fractions, which yielded nakafurans-8 (**2**, 29 mg, 1.8 mg/animal) and -9 (**3**, 32 mg, 2 mg/animal). In attempting to further purify **2** using reversed-phase HPLC (MeOH−H_2O 9:1), this labile compound degraded, preventing any further analysis. When subjected to normal-phase HPLC, the more polar crude fractions yielded variabilin (**1**) (12 mg using 6:4 hexane−EtOAc, 0.75 mg/animal), 22-deoxyvariabilin (**4**) (2.0 mg using 9:1 hexane−EtOAc, 0.13 mg/animal), and 22-deoxy-23-hydroxymethylvariabilin (**5**) (1.7 mg using 6:4 hexane−EtOAc, 0.11 mg/animal). The crude sponge extract (380 mg) was subjected to normal-phase HPLC using a mobile phase of 7:3 hexane−EtOAc, from which furospinosulin-1 (**7**) (136 mg), **1** (105 mg), **4** (6.6 mg), and **5** (5.6 mg) were obtained.

(18*R*)-Variabilin (1): colorless oil; $[\alpha]^{30}_D$ +36° (*c* 0.96, $CHCl_3$); IR (film), MS and ^1H,^{13}C NMR data are in agreement with literature values;[5] HREIMS *m*/*z* 398.2458 (calcd for $C_{25}H_{34}O_4$, 398.2457).

Nakafuran-8 (2): colorless oil; UV, IR (film), and ^1H NMR data are in agreement with literature values.[3]

Nakafuran-9 (3): colorless oil; UV, IR (film), MS, and ^1H,^{13}C NMR data are in agreement with literature values;[3] HREIMS *m*/*z* 216.1503 (calcd for $C_{15}H_{20}O$, 216.1514).

22-Deoxyvariabilin (4): colorless oil; $[\alpha]^{23}_D$ −73° (*c* 0.20, $CHCl_3$); IR (dry film) ν_{max} 1765, 1766, 1620, 850, 740 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.32 (1H, s, H-1), 7.19 (1H, s, H-4), 6.94 (1H, s, H-22), 6.26 (1H, d, J = 1 Hz, H-2), 5.15 (1H, td, J = 7, 1 Hz, H-7), 5.07 (1H, td, J = 7, 1 Hz, H-12), 4.93 (1H, d, 10 Hz, H-20), 2.85 (1H, br m, H-18), 2.43 (2H, t, J = 8 Hz, H-5), 2.23 (2H, q, J = 8 Hz, H-6), 2.05 (2H, q, J = 7 Hz, H-11), 1.98 (2H, br t, J = 7 Hz, H-10), 1.97 (3H, br s, CH_3-25), 1.94 (2H, br t, J = 7 Hz, H-15), 1.57 (3H, s, CH_3-9), 1.54 (3H, br s, CH_3-14), 1.36 (2H, br m, H-16), 1.32 (1H, br m, H-17), 1.04 (3H, d, J = 7 Hz, CH_3-19); ^{13}C NMR ($CDCl_3$), see Table 1; EIMS *m*/*z* 179 (13), 175 (17),149 (17), 137 (60), 123 (27), 95 (20), 81 (100), 69 (25), 55 (12), 41 (7); HREIMS *m*/*z* 382.2517 (calcd for $C_{25}H_{34}O_3$, 382.2508).

22-Deoxy-23-hydroxymethylvariabilin (5): yellow oil; $[\alpha]^{23}_D$ +18° (*c* 0.31, $CHCl_3$); IR (film) ν_{max} 3660−3130, 1760, 1665, 1620, 825, 772, 720 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.32 (1H, s, H-1), 7.19 (1H, s, H-4), 7.17 (1H, s, H-22), 6.26 (1H, d, J = 1 Hz, H-2), 5.15 (1H, td, J = 7, 1 Hz, H-7), 5.09 (1H, d, J = 10 Hz, H-20), 5.08 (1H, m, H-12), 4.50 (2H, br s, H-25), 2.86 (1H, br m, H-18), 2.43 (2H, t, J = 8 Hz, H-5), 2.23 (2H, q, J = 8 Hz, H-6), 2.05 (2H, q, J = 7 Hz, H-11), 1.98 (2H, br t, J = 7 Hz, H-10), 1.95 (2H, br t, J = 7 Hz, H-15), 1.58 (3H, s, CH_3-9), 1.55 (3H, br s, CH_3-14), 1.36 (2H, br m, H-16), 1.33 (2H, br m, H-17), 1.06 (3H, d, J = 7 Hz, CH_3-19); ^{13}C NMR ($CDCl_3$), see Table 1; EIMS *m*/*z* 175 (15), 153 (14), 149 (23), 135 (38), 123 (20), 109 (27), 95 (28), 81 (100), 69 (62), 55 (43), 41 (25); HREIMS *m*/*z* 398.2468 (calcd for $C_{25}H_{34}O_4$, 398.2457).

Acetylation of 5. Acetylation of **5** (1.5 mg) with pyridine (0.4 mL) and Ac_2O (0.2 mL) in the usual manner gave **6** as a yellow oil: ^1H NMR ($CDCl_3$, 400 MHz) δ 7.32 (1H, s, H-1), 7.22(1H, s, H-4), 7.19 (1H, s, H-22), 6.26 (1H, d, J = 1 Hz, H-2), 5.15 (1H, td, J = 7, 1 Hz, H-7), 5.09 (1H, d, J = 10 Hz, H-20), 5.08 (1H, m, H-12), 4.88 (2H, br s, H-25), 2.86 (1H, br m, H-18), 2.43 (2H, t, J = 8 Hz, H-5), 2.23 (2H, q, J = 8 Hz, H-6), 2.10 (3H, br s, $OOCC\mathit{H}_3$), 2.05 (2H, q, J = 7 Hz, H-11), 1.98 (H, br t, J = 7 Hz, H-10), 1.95 (2H, br t, J = 7 Hz, H-15), 1.58 (3H, s, CH_3-9), 1.55 (3H, br s, CH_3-14), 1.36 (2H, br m, H-16), 1.33 (2H, br m, H-17), 1.06 (3H, d, J = 7 Hz, CH_3-19); HREIMS *m*/*z* 440.2550 (calcd for $C_{27}H_{36}O_5$, 440.2561).

Furospinosulin-1 (7): colorless oil; IR (film), MS, and ^1H,^{13}C NMR data are in agreement with literature values;[6] HREIMS *m*/*z* 354.2933 (calcd for $C_{25}H_{38}O$, 354.2922).

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