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ScienceDirect
Tetrahedron
Tetrahedron 63 (2007) 1577–1582

Dactylospongiaquinone, a new meroterpenoid from the Australian marine sponge *Dactylospongia* n. sp.

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Received 4 October 2006; revised 20 November 2006; accepted 7 December 2006

Abstract—Chemical investigation of the sponge *Dactylospongia* n. sp. collected near Mooloolaba, S.E. Queensland, has led to the isolation of dactylospongiaquinone (**7**) together with the known quinones (**2**–**5**). The new metabolite **7** possesses a different carbon framework from the known dictyoceratidaquinone (**9**) and is suggested to possess a cis-fused ring junction by extensive NOESY studies combined with molecular modelling calculations. The relative stereochemistry of the previously described cyclospongiaquinone-1 (**3**) and dehydrocyclospongiaquinone-1 (**4**) is also assigned on the basis of NOESY analyses. Full NMR spectroscopic assignments are provided for all compounds.

1. Introduction

Sesquiterpene quinones are characteristic marine metabolites with examples of both drimane and 4,9-friedodrimane skeletons frequently isolated from sponge genera.[1] This class of compounds has attracted the attention of researchers because of their potent biological properties, which are reported to include antimicrobial,[2] antileukemic[3] and immunomodulatory activities.[4] In the late 1980s, there was significant interest in the possible anti-HIV activity of marine sesquiterpene quinones.[1,5]

Reports on these interesting compounds from Australian sponges have been limited. In 1978, the Roche group reported the five quinones, isospongiaquinone (**1**), spongiaquinone (**2**) and the cyclic compounds cyclospongiaquinone-1 (**3**), dehydrocyclospongiaquinone-1 (**4**) and cyclospongiaquinone-2 (**5**), isolated from two distinct forms of *Stelospongia conulata* collected in New South Wales.[6] Capon et al. subsequently assigned the absolute stereochemistry to spongiaquinone following its isolation from a Southern Australian *Spongia* sp. along with **4** and other sesquiterpene quinones.[7] Meanwhile, investigation of *Spongia hispida*, collected by trawling off the Victorian coast, led to the isolation of 5-*epi*-isospongiaquinone (**6**) with a biosynthetically interesting cis-fused ring junction.[8] More recent investigation of an *Eurypongia* sp. has yielded novel sesquiterpene quinone/hydroquinone pairs including some chlorinated metabolites.[9]

An ongoing screening programme for bioactive sponges collected in S.E. Queensland waters identified a dictyoceratid sponge *Dactylospongia* n. sp. that showed cytotoxic and antimicrobial activities. Chemical study of this sponge has now led to the isolation of the biosynthetically novel cyclopropyl-substituted sesquiterpene quinone, named as dactylospongiaquinone (**7**), together with the quinones (**2**–**5**). This paper reports the structural and stereochemical studies on the isolated metabolites, together with an evaluation of their biological activities.

2. Result and discussion

Extraction of the sponge sample with DCM/MeOH 1:1 gave a dark-brown extract that was fractionated by silica gel flash chromatography (hexanes/DCM ⇒ DCM ⇒ MeOH), followed by preparative TLC using hexanes/DCM 1:4 to give spongiaquinone (**2**), cyclospongiaquinone-1 (**3**), dehydrocyclospongiaquinone-1 (**4**) and cyclospongiaquinone-2 (**5**), all identified by comparison with literature data.[6,7] In the course of this work, one of the less polar column fractions attracted our attention since its ^{1}H NMR spectrum showed doublet signals at δ_H 0.50 and −0.08 that were diagnostic of cyclopropyl ring protons. This fraction was further

Keywords: Meroterpenoid; Quinone; Cyclopropane; NMR; Sponges; *Dactylospongia*.
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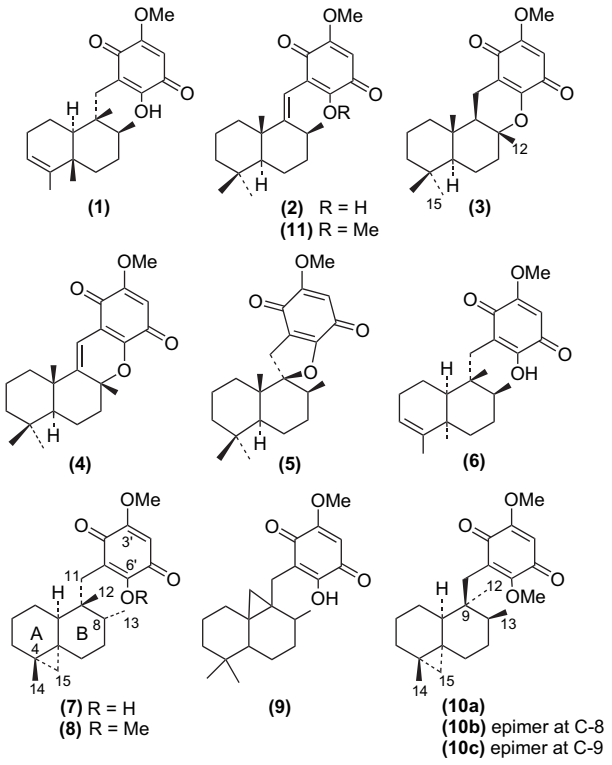
doi:10.1016/j.tet.2006.12.006

investigated by preparative TLC and then by silica gel HPLC using hexanes/EtOAc 7:3. The HPLC isolation of the new compound **7** was complicated by the co-elution of related metabolites,[7] hence the sample was converted to its methyl ether **8** and then subjected to further HPLC purification. Both **7** and **8** were then subjected to a detailed NMR study at 750 MHz.



The molecular formula of dactylospongiaquinone (**7**) was established as $C_{22}H_{30}O_4$ based on high-resolution mass measurement. ^{13}C NMR showed diagnostic signals for a hydroxy quinone at δ_C 181.9, 181.8, 161.0, 152.9, 117.8 and 101.9, and a methoxyl carbon at δ_C 56.7, while the ^1H NMR spectrum showed a sole quinonoid proton at δ_H 5.83 and a methoxyl group at δ_H 3.83. In the sesquiterpene portion, ^{13}C NMR data showed the presence of three methyls, seven methylenes, two methines and three quaternary carbons (Table 1). There were two methyl singlets at δ_H 1.04 and 0.80, and a methyl doublet at δ_H 0.93 (3H, d, J=6.8 Hz) linked to a multiplet at δ_H 1.37. There were two isolated AB systems, one at δ_H 2.73 and 2.46 (1H each, d, J=13.5 Hz; δ_C 32.9) adjacent to the quinone ring,[9] and the other at δ_H 0.50 and −0.08 (1H each, d, J=4.2 Hz; δ_C 22.4) for the cyclopropyl ring. HMBC correlations from the cyclopropyl protons identified the two other cyclopropyl carbons, at δ_C 20.0 and 24.0. The 2D data set acquired for dactylospongiaquinone was then assessed against two carbon skeletons **7** and **9**, which each contains two methyl singlets and a methyl doublet, but which differ in the positioning of the cyclopropyl group. Dictyoceratidaquinone (**9**) is a cyclopropyl-containing sesquiterpene quinone of undetermined stereochemistry that was isolated by Utkina and Veselova from an undescribed dictyoceratid sponge collected from the Indian Ocean.[10] This group reported cyclopropyl signals at δ_H 0.53 and 0.31, and also δ_C 16.8 (t), 27.1 (s) and 28.0 (s) for **9**. It was apparent from these data that the *Dactylospongia* cyclopropyl compound was not dictyoceratidaquinone. Furthermore, HMBC correlations from the methyl singlet at δ_H 0.80 to C-11 at δ_C 32.9 required this methyl group to be placed at C-9, consistent with structure **7**. HMBC correlations from the methyl singlet at δ_H 1.04 to the carbons at δ_C 20.0, 22.4 and 24.0, and from the cyclopropyl protons at δ_H 0.50 and −0.08 to the signal at δ_C 20.2 then placed a methyl group next to the cyclopropyl ring. TOCSY data indicated the partial structure H-6 to H-8 to Me-13. These results, together with data for the ^1H and ^{13}C NMR signals of the related dimethyl ether **8** (Table 1), supported the planar structure for **7** as shown.

The relative configuration of dactylospongiaquinone was next explored. Two pieces of evidence strongly supported a cis-fused ring junction. Firstly, there was poor agreement of the ^{13}C NMR data for C-3–C-10 and C-15 with the corresponding data for the cyclopropane-containing terpenes cacospongionolide,[11] asmarines I and J[12] and the dytesinins A and B.[13] All these metabolites are suggested to be trans ring fused by extensive NOE studies. Secondly, in the NOESY data for methyl ether **8**, a strong correlation between the cyclopropyl proton at δ_H 0.47 and a proton at δ_H 1.340, assigned to H-10, could only be consistent with cis stereochemistry. Likewise, an NOE between H-10 (δ_H 1.39) and a cyclopropyl proton (δ_H 0.50) was apparent in compound **7**.

Molecular modelling linked with NOESY data provided insights into the stereochemistry at C-8 and C-9. Using the program PC-Model,[14] a conformational search was carried out on each of the four possible cis-fused diastereomers (**8**, **10a**–**10c**). Figure 1 shows the lowest energy conformation for each stereoisomer, although, it should be noted that each isomer showed a number of conformations for both fused ring system and rotatable side chains. Proton–proton distances were then averaged (weighted by population) over all significant conformations. The NOEs observed between Me-12 and the individual methylene protons at C-1 of dactylospongiaquinone methyl ether were more consistent with structure **8** or **10c** than with structure **10a** or **10b**, in which the C-9 stereochemistry is inverted. In both structures **8** and **10c**, ring B adopts a chair conformation in the lowest energy conformation, and with the quinone substituent axially orientated. The observed NOEs between the H-11 protons and the H-7 signal at δ_H 1.70 were entirely consistent with this conformational picture. The Me-13 group showed NOE correlations to the H-11 protons, and so could be placed on the same face as the quinone group. Consistent with its equatorial position, Me-13 showed NOE correlations to both H-7 protons, and there was also a strong NOE from Me-12 (δ_H 0.80) to Me-13 (δ_H 0.93). The NOE correlations reported in Table 1 for compound **8** all corresponded to proton–proton distances of 3.5 Å or less in one or more low energy conformations.

We then considered the relative stereochemistry of the two cyclospongiaquinone-1 metabolites **3** and **4**, since this had not been evaluated in the original Roche study.[6] For cyclospongiaquinone-1 (**3**), the methyl group at δ_H 0.86 assigned to H-12 showed a strong NOE to the methyl singlet at δ_H 1.20 for H-13, therefore, these two groups were *syn*. Likewise for the dehydro compound **4**, the observation of an

Table 1. NMR assignments for compounds **7** and **8**

Dactylospongiaquinone **7**			Dactylospongiaquinone methyl ether **8**			
Carbon	δ_C[a]	δ_H[b]	δ_C[a]	δ_H[b]	HMBC	Selected NOESY
1	20.3 (t)	1.63 (1H, m), 1.48 (1H, m)	20.4 (t)	(a) 1.55 (1H, m), (b) 1.43 (1H, m)	H-10	—
2	18.2 (t)	1.52 (1H, m), 1.36 (1H, m)	18.2 (t)	(a) 1.52 (1H, m), (b) 1.39 (1H, m)	H-1,[c] H-3	—
3	29.3 (t)	1.50 (1H, m), 1.42 (1H, m)	29.0 (t)	β 1.52 (1H, m), α 1.40 (1H, m)	H-2	H-14; H-15a
4	20.0 (s)	—	19.8 (s)	—	H-3, H-14, H-15	—
5	24.0 (s)	—	23.8 (s)	—	H-6, H-10, H-14, H-15	—
6	29.2 (t)	1.50 (1H, m), 1.16 (1H, m)	28.4 (t)	(a) 1.50 (1H, m), (b) 1.14 (1H, m)	H-7, H-8, H-15	H-14, H-15b; H-14, H-15b
7	29.9 (t)	1.67 (1H, m), 1.18 (1H, m)	29.7 (t)	α 1.70 (1H, m), β 1.17 (1H, m)	H-6, H-8, H-13	H-11,[c] H-13; H-8, H-13
8	37.0 (d)	1.37 (1H, m)	36.8 (d)	1.345[d] (1H, m)	H-7, H-11, H-12, H-13	H-7β, H-12, H-13
9	32.1 (s)	—	32.9 (s)	—	H-8, H-10, H-11, H-12, H-13	—
10	43.0 (d)	1.39 (1H, m)	43.2 (d)	1.340[d] (1H, m)	H-1α, H-6, H-11, H-12, H-15	H-11, H-12, H-15a
11	32.9 (t)	2.73 (1H, d, J=13.5 Hz), 2.46 (1H, d, J=13.5 Hz)	33.1 (t)	2.76 (1H, d, J=13.0 Hz), 2.44 (1H, d, J=13.0 Hz)	H-8, H-10, H-12	H-7α, H-10, H-12, H-13; H-7α, H-10, H-12, H-13
12	18.1 (q)	0.80 (3H, s)	18.2 (q)	0.77 (3H, s)	H-8, H-10, H-11, H-13	H-1, H-11, H-13
13	17.5 (q)	0.93 (3H, d, J=6.8 Hz)	17.5 (q)	0.91 (3H, d, J=6.8 Hz)	H-7, H-8, H-11, H-12	H-7, H-8, H-11b, H-12
14	20.2 (q)	1.04 (3H, s)	20.2 (q)	1.04 (3H, s)	H-3, H-15	H-3, H-6, H-15b
15	22.4 (t)	(a) 0.50 (1H, d, J=4.2 Hz), (b) −0.08 (1H, d, J=4.2 Hz)	22.6 (t)	(a) 0.47 (1H, d, J=4.2 Hz), (b) −0.06 (1H, d, J=4.2 Hz)	H-6, H-10, H-14	H-3α, H-10, H-15b; H-6, H-14, H-15a
1′	117.8 (s)	—	129.2 (s)	—	H-11	—
2′	181.8 (s)	—	182.5 (s)	—	H-11, H-4′	—
3′	161.0 (s)	—	158.9 (s)	—	H-4′, 3′-OMe	—
4′	101.9 (d)	5.83 (1H, s)	105.0 (d)	5.72 (1H, s)	—	3′-OMe
5′	181.9 (s)	—	183.2 (s)	—	H-4′	—
6′	152.9 (s)	—	157.0 (s)	—	H-11, H-4′, 6′-OMe	—
3′-OMe	56.7 (q)	3.83 (3H, s)	56.7 (q)	3.80 (3H, s)	—	H-4′
6′-OH	—	7.28 (1H, br s)			—	
6′-OMe	—	—	60.7 (q)	4.10 (3H, s)	—	—

[a] Chemical shifts (ppm) referenced to $CDCl_3$ (δ_C 77.0), 750 MHz.
[b] Chemical shifts (ppm) referenced to $CHCl_3$ (δ_H 7.25), 750 MHz.
[c] Correlations to both methylene protons unless specified.
[d] Distinguishable at 750 MHz.

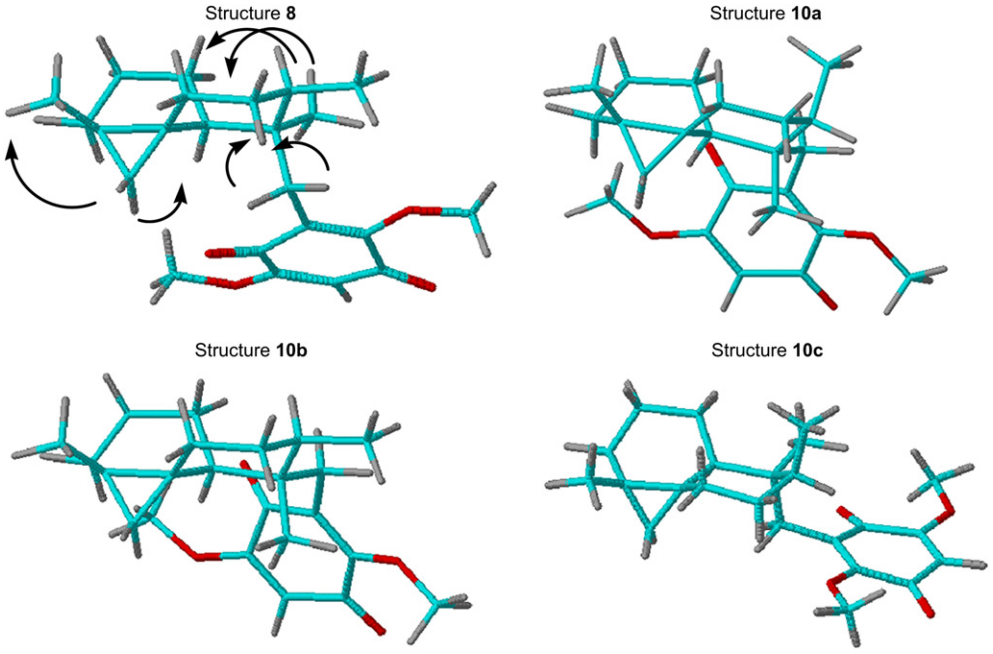


Figure 1. Lowest energy conformation for candidate structures of dactylospongiaquinone methyl ether.

NOE from H-12 to H-13 supported the *syn* relationship. HSQC and HMBC correlations were used to assign fully the NMR data for **3–5** as presented in Section 3.

Owing to its unusual chiroptic properties, the $[\alpha]_D$ value of spongiaquinone (**2**) is not recorded in the literature,[6,7] however, for (−)-spongiaquinone methyl ether (**11**) the absolute stereochemistry shown has been established by chemical correlation[7] and by total synthesis.[15] A sample of **11** prepared from spongiaquinone (**2**) isolated in this study had an $[\alpha]_D$ value of −91.3 (*c* 0.35) compared to the value of −82.2 (*c* 0.52) reported by Capon et al.[7]

Both trans- and cis-fused drimane and 4,9-friedodrimane metabolites have been isolated from marine sponges.[1,16] A biosynthetic scheme leading to dactylospongiaquinone is shown in Figure 2, which may suggest a role for the carbocationic intermediate **12**. The stereochemistry at C-9 and C-10 results from the methyl and hydride migrations shown, while that at C-8 is of interest as it mirrors the stereochemistry found in the asmarines I and J.[12]

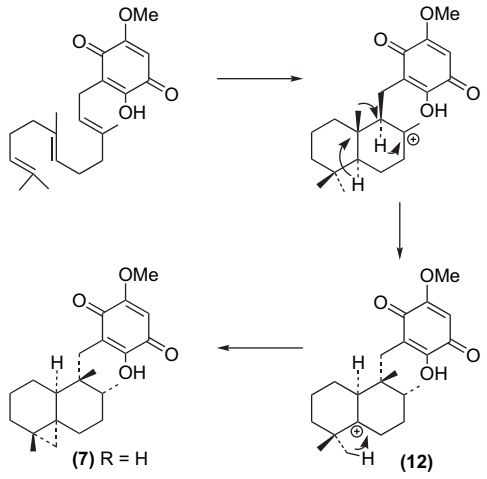


Figure 2. Suggested biosynthetic pathway leading to dactylospongiaquinone **7**.

Spongiaquinone (**2**) exhibited strong cytotoxic activity against human breast cancer (BC) cells (IC_{50} 3.24 μg/ml), whereas cyclospongiaquinone-2 (**5**) showed moderate activity against human small cell lung cancer (NCI-H187) cells (IC_{50} 4.96 μg/ml). Both compounds were inactive in antitubercular and antimalarial screens.

3. Experimental

3.1. General experimental procedures

Optical rotations were obtained using a JASCO-P1010 polarimeter. 1D and 2D NMR spectra were acquired using Bruker DRX-500 or Bruker DMX-750 instrument. NMR spectra were obtained in deuterochloroform at room temperature. Samples were internally referenced to $CHCl_3$ at δ_H 7.25 or $CDCl_3$ at δ_C 77.0. High- and low-resolution mass measurements were obtained from a Finnigan MAT 900 XL-Trap electrospray (ESI) mass spectrometer with a Finnigan API III electrospray source.

3.2. Biological material

Specimens of *Dactylospongia* n. sp. were collected from the Inner Gneerings, a group of shoals near Mooloolaba (Australia), using SCUBA at a depth of 10–15 m on 16 January 2006. Samples were taken back to the laboratory where they were stored at −20 °C until extraction. The sponge was charcoal grey on the surface and orange-yellow on the underside. The shape was globular and the sample was approximately 10 cm thick. A voucher specimen (QM G324323) is lodged at the Queensland Museum. Photographs of the sponge material are available from the authors.

3.3. Extraction and isolation of quinones

The specimen of *Dactylospongia* n. sp. (wet weight 163 g) was cut into small pieces and extracted exhaustively with MeOH. The extract was removed, filtered through cotton and then evaporated under reduced pressure to give an aqueous residue, which was partitioned sequentially with hexanes, DCM, EtOAc and finally *n*-BuOH. The hexane fraction was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give 960 mg of a brown solid, which was analysed by TLC and ^{1}H NMR. The extract was subjected to gradient elution Si flash chromatography (hexanes⇒DCM⇒MeOH). The fractions that eluted in hexanes/DCM (1:3) were combined and analysed by TLC and ^{1}H NMR. The EtOAc/hexanes (3:7) soluble portion of this fraction was purified using semi-preparative NP-HPLC (Waters 515; Gilson 132 series RI detector; Waters 10μ μPorasil 7.8×300 mm column; flow rate 2.2 ml/min) with EtOAc/hexanes (3:7) as solvent to afford **7**. The fractions that eluted in hexanes/DCM (1:4) were combined and analysed by TLC and ^{1}H NMR yielding spongiaquinone (**2**), cyclospongiaquinone-1 (**3**), dehydrocyclospongiaquinone-1 (**4**) and cyclospongiaquinone-2 (**5**).

3.3.1. Spongiaquinone (2).[6,7] Compound **2** (1.20 mg) was obtained as a red amorphous solid: $[\alpha]_D$ −50.0 (*c* 0.07, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz) δ_H 5.85 (1H, s, H-4′), 5.53 (1H, s, H-11), 3.83 (3H, s, OMe), 2.52 (1H, m, H-8), 1.80 and 1.49 (1H each, m, H-1), 1.70 and 1.45 (1H each, m, H-2), 1.55 (2H, m, H-6), 1.54 (2H, m, H-7), 1.37 and 1.22 (1H each, m, H-3), 1.19 (3H, s, H-13), 1.09 (3H, d, *J*=7.0 Hz, H-12), 0.87 (3H, s, H-14) and 0.87 (3H, s, H-15); ^{13}C NMR ($CDCl_3$, 500 MHz) δ_C 181.5 (C, C-5′), 181.0 (C, C-2′), 162.9 (C, C-9), 161.1 (C, C-3′), 150.0 (C, C-6′), 117.0 (C, C-1′), 106.8 (CH, C-11), 102.1 (CH, C-4′), 56.5 (OMe-3′), 54.3 (C, C-5), 41.9 (CH_2, C-3), 41.1 (C, C-10), 38.3 (CH_2, C-1), 33.9 (C, C-4), 33.8 (CH_3, C-15), 33.7 (CH_2, C-7), 33.1 (CH, C-8), 22.3 (CH_3, C-13), 21.6 (CH_3, C-12), 21.5 (CH_3, C-14), 18.8 (CH_2, C-2) and 17.6 (CH_2, C-6); LRESIMS *m*/*z* 381 $(M+Na)^+$.

3.3.2. Cyclospongiaquinone-1 (3).[6] Compound **3** (1.30 mg) was obtained as a yellow amorphous solid: $[\alpha]_D$ +94.6 (*c* 0.06, $CHCl_3$), lit.[6] $[\alpha]_D$ −2.15 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz) δ_H 5.73 (1H, s, H-4′), 3.78 (3H, s, OMe), 2.49 and 2.06 (1H each, m, H-11), 2.20 and 1.76 (1H each, m, H-1), 1.75 and 1.31 (1H each, m, H-6), 1.72 and 0.75 (1H each, m, H-7), 1.60 and 1.45 (1H each, m, H-2), 1.42 (1H, dd, *J*=18.0, 4.0 Hz, H-9), 1.20 (3H, s, H-13), 0.97 (1H, m, H-5), 0.87 (3H, s, H-14), 0.86 (3H, s,

H-12) and 0.81 (3H, s, H-15); ^{13}C NMR ($CDCl_3$, 500 MHz) δ_C 181.2 (C, C-5′), 181.0 (C, C-2′), 161.1 (C, C-3′), 152.0 (C, C-6′), 116.0 (C, C-1′), 104.5 (CH, C-4′), 81.7 (C, C-8), 56.2 (OMe-3′), 55.9 (CH, C-5), 51.2 (CH, C-9), 41.6 (CH_2, C-3), 40.1 (CH_2, C-1), 39.1 (CH_2, C-7), 37.0 (C, C-10), 33.3 (CH_3, C-15), 33.0 (C, C-4), 21.4 (CH_3, C-14), 20.3 (CH_3, C-13), 19.6 (CH_2, C-6), 18.4 (CH_2, C-2), 16.1 (CH_2, C-11) and 14.9 (CH_3, C-12); LRESIMS *m/z* 381 $(M+Na)^+$.

3.3.3. Dehydrocyclospongiaquinone-1 (4).[6] Compound **4** (0.40 mg) was obtained as an orange amorphous solid: sample decomposed before an $[\alpha]_D$ value could be measured; ^{1}H NMR ($CDCl_3$, 500 MHz) δ_H 6.24 (1H, s, H-11), 5.73 (1H, H-4′), 3.79 (3H, s, OMe), 2.33 and 1.94 (1H each, m, H-7), 1.98 and 1.35 (1H each, m, H-1), 1.64 (2H, m, H-6), 1.60 and 1.14 (1H each, m, H-2), 1.49 (3H, s, H-13), 1.40 and 1.12 (1H each, m, H-3), 1.13 (3H, s, H-12), 1.03 (1H, m, H-5), 0.89 (3H, s, H-14) and 0.84 (3H, s, H-15); ^{13}C NMR ($CDCl_3$, 500 MHz) δ_C 180.0 (C, C-5′), 179.0 (C, C-2′), 158.8 (C, C-3′), 151.6 (C, C-9), 149.4 (C, C-6′), 118.0 (C, C-1′), 106.8 (CH, C-11), 104.9 (CH, C-4′), 82.8 (C, C-8), 56.3 (OMe-3′), 51.6 (CH, C-5), 41.1 (CH_2, C-3), 41.0 (CH_2, C-7), 39.7 (C, C-10), 37.8 (CH_2, C-1), 33.4 (C, C-4), 33.0 (CH_3, C-15), 26.8 (CH_3, C-13), 23.4 (CH_3, C-12), 21.6 (CH_3, C-14), 21.5 (CH_2, C-6), 18.8 (CH_2, C-6) and 18.6 (CH_2, C-2); LRESIMS *m/z* 379 $(M+Na)^+$.

3.3.4. Cyclospongiaquinone-2 (5).[6] Compound **5** (1.3 mg) was obtained as an orange amorphous solid: $[\alpha]_D$ −18.1 (*c* 0.15, $CHCl_3$), lit.[6] $[\alpha]_D$ +11.68 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz) δ_H 5.67 (1H, s, H-4′), 3.83 (3H, s, OMe), 3.38 and 2.64 (1H each, d, *J*=17.0 Hz, H-11), 2.06 (1H, m, H-8), 1.62 (2H, m, H-7), 1.61 and 1.50 (1H each, m, H-2), 1.46 (2H, m, H-6), 1.35 and 1.10 (1H each, m, H-1), 1.25 and 1.14 (1H each, m, H-3), 1.18 (3H, s, H-12), 1.18 (3H, d, *J*=7.0 Hz, H-13), 0.97 (1H, m, H-5), 0.89 (3H, s, H-15) and 0.87 (3H, s, H-14); ^{13}C NMR ($CDCl_3$, 500 MHz) δ_C 179.0 (C, C-2′), 178.6 (C, C-5′), 160.8 (C, C-3′), 159.2 (C, C-6′), 118.1 (C, C-1′), 104.1 (CH, C-4′), 103.1 (C, C-9), 56.7 (OMe-3′), 48.7 (CH, C-5), 43.3 (C, C-10), 41.6 (CH_2, C-1), 40.8 (CH, C-8), 40.5 (CH_3, C-13), 36.8 (CH_2, C-11), 33.2 (CH_3, C-15), 32.9 (C, C-4), 31.8 (CH_2, C-3), 29.1 (CH_2, C-7), 21.1 (CH_3, C-14), 16.8 (CH_2, C-2), 16.6 (CH_2, C-6) and 16.6 (CH_3, C-12); LRESIMS *m/z* 381 $(M+Na)^+$.

3.3.5. Dactylospongiaquinone (7). Compound **7** (0.58 mg) was obtained as a yellow amorphous solid: $[\alpha]_D$ −27.9 (*c* 0.03, $CHCl_3$); ^{1}H and ^{13}C NMR ($CDCl_3$, 750 MHz) see Table 1; HRESIMS *m/z* 381.2036, calcd for $C_{22}H_{30}O_4Na$ 381.2042.

3.3.6. Dactylospongiaquinone methyl ether (8). A sample of **7** (1.8 mg, 0.005 mmol) was stirred with MeI (0.47 µl, 0.0075 mmol, 1.5 equiv) and K_2CO_3 (0.76 mg, 0.0055 mmol, 1.1 equiv) in anhydrous DMF (20 µl) at room temperature overnight. The reaction mixture was diluted with H_2O and extracted with CH_2Cl_2 (3×5 ml). The organic layer was washed with H_2O and dried (Na_2SO_4). After filtration through a cotton wool plug and removal of the solvent, the crude methyl ether was further purified by semi-preparative NP-HPLC (20% EtOAc/hexanes, 1.5 ml/min (2:8)) to afford **8** (0.32 mg, 17%) as a yellow amorphous solid: $[\alpha]_D^{22}$ −16.4 (*c* 0.05, $CHCl_3$); for ^{1}H and ^{13}C NMR ($CDCl_3$, 750 MHz) data see Table 1; HRESIMS *m/z* 395.2205, calcd for $C_{23}H_{32}O_4Na$ 395.2198.

3.3.7. Spongiaquinone methyl ether (11). Compound **11** was prepared similarly as a yellow oil: $[\alpha]_D$ −91.3 (*c* 0.35, $CHCl_3$), lit.[7] $[\alpha]_D$ −82.2 (*c* 0.52, $CHCl_3$); NMR data were in accordance with literature values.[7]

3.4. Cytotoxicity assays

The cytotoxicity assays against BC and NCI-H187 cells were performed employing a colourimetric method.[17] The standard drug ellipticine exhibited IC_{50} values against these cell lines at 1.46 and 0.39 µg/ml, respectively.

Acknowledgements

We thank the Royal Golden Jubilee Fund, Thailand for a Ph.D. scholarship (to A.J.), the Australia Research Council for funding, L. Lambert (Centre for Magnetic Resonance, UQ), G. MacFarlane of the School of Molecular and Microbial Science, UQ, for spectroscopic assistance, Professor R. Capon, Institute for Molecular Bioscience, UQ, for assistance with the measurements of $[\alpha]_D$ values, and K. Rands-Trevor for her assistance with the preparation of compound **12**. ScubaWorld Mooloolaba assisted with access to dive sites for the sample collection (carried out under permit from the Department of Primary Industries and Fisheries, Queensland).

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