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Stereochemical evaluation of sesquiterpene quinones from two sponges of the genus *Dactylospongia* and the implication for enantioselective processes in marine terpene biosynthesis

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ABSTRACT

Silver nitrate flash chromatography of the organic extract from the sponge *Dactylospongia elegans* has led to the isolation of three new sesquiterpene quinones isohyatellaquinone (**7**), 7,8-dehydrocyclospongiaquinone-2 (**8**) and 9-*epi*-7,8-dehydrocyclospongiaquinone-2 (**9**) together with the known quinones dictyoceratidaquinone (**6**), mamanuthaquinone (**10**), ilimaquinone (**11**), hyatellaquinone (**12**) and the sesterterpene furospinosulin (**22**). The relative stereochemistry of dictyoceratidaquinone (**6**) is assigned on the basis of NOESY analysis. A second species of *Dactylospongia*, thought to be new to science, was found to contain *ent*-(**7**) together with the new quinone neomamanuthaquinone (**13**). The isolation of antipodal sesquiterpenes from closely related species has implications for the stereochemical evaluation of terpene metabolites. The biosynthetic processes in these marine sponges may involve terpene synthases that do not discriminate chiral substrates or may result from the presence of multiple terpene synthases, each with differing enantioselectivity.

1. Introduction

In the marine literature, there are many examples of sesquiterpene quinone metabolites that have been isolated from sponges and algae. Reported biological activities that include antimicrobial, antileukaemic, antiviral and immunomodulatory effects have led to a wealth of metabolites possessing either the regular drimane skeleton or the rearranged 4,9-friedodrimane skeleton.[1] Recently our screening programme of marine sponges from South East Queensland uncovered a new sponge species of the genus *Dactylospongia*, the crude organic extract of which contained signals characteristic of a cyclopropyl motif. This led us to isolation of the novel cyclopropyl-containing dactylospongiaquinone (**1**) that possessed the biosynthetically interesting cis ring junction (rather than the more commonly encountered trans stereochemistry). The new quinone **1** was isolated together with the frequently encountered bioactive quinones spongiaquinone (**2**), cyclospongiaquinone-1 (**3**), dehydrocyclospongiaquinone-2 (**4**) and cyclospongiaquinone-2 (**5**).[2] Prior to our study, the only previous report of cyclopropyl functionality in sponge quinone metabolites was the poorly characterised dictyoceratidaquinone (**6**) reported by Utkina and Veselova from a dictyoceratid sponge.[3] With the data of dactylospongiaquinone in mind, we prioritised chemical study of a second sponge sample that showed NMR signals suggestive of a cyclopropyl-functionalised quinone. From this sample, which was identified as *Dactylospongia elegans*, we now report the isolation of dictyoceratidaquinone (**6**)[3] together with three new sesquiterpene quinones **7**–**9** and the three known quinones **10**–**12**.[4–6] The sponge was collected at the same underwater location as the earlier studied *Dactylospongia* species which we have now found contains *ent*-(**7**) and the new quinone **13** as minor components. During the chemical investigation, we found that separation of structurally related sesquiterpene quinone components was facilitated by use of silver nitrate-impregnated silica as a support during chromatography.

2. Results and discussion

2.1. Structural studies

Sponge samples were collected by SCUBA from the Gneerings Reef offshore from Mooloolaba in South East Queensland; this is

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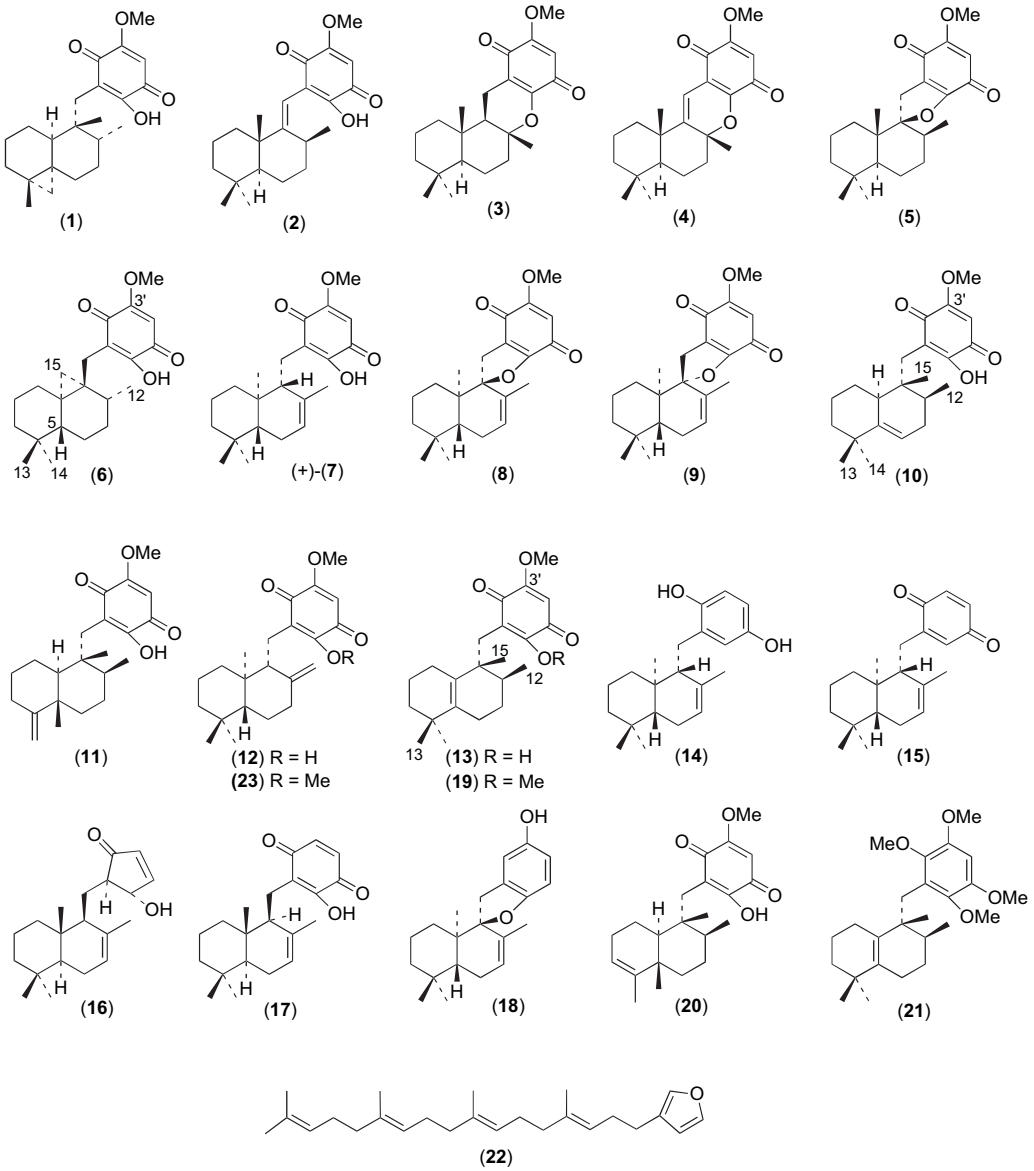

a subtropical reef that contains a mixing of tropical and temperate faunas. Extraction of the *D. elegans* sample with CH_2Cl_2/MeOH 1:1 gave a dark-brown extract that was fractionated by silica gel flash chromatography (hexanes/CH_2Cl_2 → CH_2Cl_2 → EtOAc → MeOH), followed by preparative TLC on $AgNO_3$-impregnated silica using hexanes/EtOAc. $AgNO_3$–Si chromatography is documented to facilitate separation of compounds containing alkene or aromatic units through the formation of silver ion complexes onto the π double bond. The technique is particularly suitable for separation of compounds that differ in alkene substitution pattern and generally follows an elution profile in which the least substituted alkene is eluted later compared to a more substituted alkene ($RCH{=}CH_2 > R_2C{=}CH_2 > R_2C{=}CHR > R_2C{=}CR_2$).[7]

Using these separation protocols, the three known metabolites were identified by comparison of NMR data with the literature. These were (−)-mamanuthaquinone (**10**),[4] for which our NMR data were in accordance with literature values except for C-12 and C-14, and also (−)-ilimaquinone (**11**)[5] and (+)-hyatellaquinone (**12**).[6]

A flash column fraction that contained mixed quinone components showed proton NMR signals at δ_H 0.29 and 0.52 that were diagnostic for the cyclopropyl group of dictyoceratidaquinone (**6**).[3] Following preparative TLC on silver nitrate-impregnated silica, a 2D study (500 MHz) confirmed the isolation of dictyoceratidaquinone. Assignments of proton and carbon signals are shown in Table 1. The earlier Russian work left aspects of the structure of **6** unresolved, including the positioning of the cyclopropyl ring and the relative configuration. Our gHMBC data showed correlations from the cyclopropyl (H-15) protons to C-1, C-5, C-8, C-9, C-10 and C-11, and are fully consistent with the structure shown. Selective NOE correlations from the cyclopropyl proton H-15 at δ_H 0.52 to the methyl doublet at δ_H 0.64, assigned to Me-12, and to the axial methyl at δ_H 0.81, assigned to Me-14, showed that these three groups were *syn*. The ring junction between C-5 and C-10 was trans as this places H-15 and Me-14 within 3 Å of each other; in the alternative cis conformation, the axial Me-14 is >5 Å away from the cyclopropyl proton. Selective NOE correlations from the methyl doublet for Me-12 were observed to H-6ax at δ_H 0.70, to H-8 and to H-15. No $[\alpha]_D$ value was obtained as **6** decomposed prior to optical measurement.

The three new quinones that were isolated were all subjected to detailed NMR study at 500 MHz. The molecular formula of quinone **7** was established as $C_{22}H_{30}O_4$ based on high-resolution mass measurement. ^{13}C NMR showed diagnostic signals for a hydroxyl quinone at δ_C 181.8, 181.7, 161.1, 151.2, 120.7 and 101.8, with a methoxyl carbon at δ_C 56.6; the corresponding ^1H NMR showed

Table 1
NMR assignments for compounds **6**–**9**, and **13**

#	Compound **6**		Compound **7**		Compound **8**		Compound **9**		Compound **13**	
	δ_C[a]	δ_H[b]	δ_C[a]	δ_H[b]	δ_C[a]	δ_H[b]	δ_C[a]	δ_H[b]	δ_C[c]	δ_H[d]
1	33.7	1.46 (1H, m), 1.59 (1H, m)	39.1	1.26 (1H, ddd, 13.1, 13.1, 3.7), 1.86 (1H, m)	30.7	1.30 (1H, m), 1.34 (1H, m)	30.5	1.23 (1H, m), 1.31 (1H, m)	25.5	1.38 (1H, m), 2.10 (1H, m)
2	21.9	1.4–1.6 (2H)	19.0	1.42 (1H, m), 1.52 (1H, m)	17.9	1.45 (1H, m), 1.54 (1H, m)	17.7	1.41 (1H, m), 1.54 (1H, m)	20.0	1.61 (1H, m)
3	42.6	1.15 (1H, m), 1.35 (1H, m)	42.0	1.14 (1H, ddd, 13.0, 13.3, 3.0), 1.37 (1H, m)	41.2	1.21 (1H, ddd, 13.4, 13.4, 3.5), 1.37 (1H, m)	41.7	1.13 (1H, m), 1.42 (1H, m)	40.0	1.37 (1H, m), 1.40 (1H, m)
4	34.9		33.1	—	32.6	—	32.6	—	34.5	—
5	48.3	1.30 (1H, m)	50.0	1.21 (1H, dd, 12.1, 4.8)	42.3	1.82 (1H, dd, 12.1, 4.7)	43.7	1.36 (1H, dd, 11.3, 5.6)	135.0	—
6	16.8	0.70 ax, 1.28 eq	23.7	1.83 (1H, m), 1.92 (1H, m)	23.9	1.90 (1H, m), 2.10 (1H, m)	23.8	1.95 (1H, m), 2.00 (1H, m)	25.5	1.88 (1H, m), 2.02 (1H, m)
7	30.6	1.07 (1H, m), 1.25 (1H, m)	122.6	5.37 (1H, br s)	129.6	5.69 (1H, br s)	122.7	5.38 (3H, br s)	21.5	1.88 (1H, m), 2.10 (1H, m)
8	29.0	1.96 (1H, m)	134.9	—	130.3	—	135.6	—	34.8	1.52 (1H, m)
9	26.8	—	51.5	2.42 (1H, m)	101.5	—	102.3	—	43.0	—
10	27.6	—	37.1	—	40.9	—	41.5	—	131.5	—
11	28.9	2.39 (1H, d, 14.1), 2.65 (1H, d, 14.1)	21.4	2.46 (2H, m)	28.7	3.03 (1H, d, 17.2), 2.88 (1H, d, 17.2)	35.4	2.75 (1H, d, 17.3), 3.31 (1H, d, 17.3)	32.3	2.54 (1H, d, 13.1), 2.68 (1H, d, 13.1)
12	19.8	0.64 (3H, d, 7.0)	22.1	1.55 (3H, m)	18.4	1.63 (3H, m)	17.2	1.62 (3H, m)	15.4	0.76 (3H, d, 6.9)
13	30.9	0.84 (3H, s)	33.2	0.83 (3H, s)	32.5	0.89 (3H, s)	32.8	0.86 (3H, s)	28.0	0.97 (3H, s)
14	20.4	0.81 (3H, s)	22.0	0.87 (3H, s)	22.0	0.91 (3H, s)	21.6	0.93 (3H, s)	29.6	0.93 (3H, s)
15	18.0	0.29 (1H, 5.0), 0.52 (1H, 5.0)	13.3	0.84 (3H, s)	15.2	0.90 (3H, s)	15.4	1.12 (3H, s)	22.0	0.81 (3H, s)
1′	118.6	—	120.7	—	118.3	—	118.4	—	118.0	—
2′	182.0	—	181.7	—	178.1	—	177.9	—	182.5	—
3′	161.2	—	161.1	—	160.8	—	160.7	—	161.5	—
4′	102.0	5.84 (1H, s)	101.8	5.82 (1H, s)	103.9	5.66 (1H, s)	104.3	5.66 (1H, s)	105.0	5.83 (1H, s)
5′	182.0	—	181.8	—	179.4	—	179.2	—	183.0	—
6′	152.2	—	151.2	—	159.4	—	159.9	—	153.5	—
3′-OMe	56.8	3.85 (3H, s)	56.6	3.84 (3H, s)	56.6	3.81 (3H, s)	56.8	3.81 (3H, s)	56.3	3.84 (3H, s)
6′-OH		—[e]		7.37 (1H, s)		—		—		7.31 (1H, s)

[a] Frequency 500 MHz; chemical shifts (ppm) referenced to $CDCl_3$ (δ_C 77.0).
[b] Frequency 500 MHz; chemical shifts (ppm) referenced to $CHCl_3$ (δ_H 7.24).
[c] Frequency 100 MHz; chemical shifts (ppm) referenced to $CDCl_3$ (δ_C 77.0).
[d] Frequency 400 MHz; chemical shifts (ppm) referenced to $CHCl_3$ (δ_H 7.24).
[e] Not observed.

a sole quinonoid proton at δ_H 5.82, a methoxyl group at δ_H 3.84 and an exchangeable hydroxyl proton at δ_H 7.37 in $CDCl_3$. The remaining signals from the ^{13}C NMR were all attributed to the sesquiterpene moiety containing a methyl substituted olefinic double bond (δ_C 122.6 (d) and 134.9 (s)). There were three methyl singlets at δ_H 0.83, 0.84 and 0.87, a vinylic methyl singlet at δ_H 1.55, one olefinic proton at δ_H 5.37, plus a complex 2H signal at δ_H 2.46. In the HMBC spectrum, the methylene protons at δ_H 2.46 showed correlations to the quinone ring (C-1′, C-2′ and C-6′) and also to C-8, C-9 and C-10 of the drimane moiety, and so were assigned to H-11 even though the signal lacked the characteristic double doublet pattern commonly associated with the C-11 methylene protons of sesquiterpene quinones.[2–6] The NMR assignments were all closely similar to those reported for the drimane moiety of isozonarol (**14**),[8] isozonarone (**15**)[8] and dysienone (**16**),[9] and most notably for the sesquiterpene **17** from *Dysidea* cf. *cristagalli*, which showed similar assignments for the C-11 position (δ_H 2.46–2.54 (m) and δ_C 21.6).[10] The complex splitting pattern for H-11 was resolved by rerunning the spectroscopic data of **7** in C_6D_6, which revealed a regular AB system at δ_H 2.53 and 2.67 (1H each, dd, J=13.6, 3.4 Hz), with each proton showing HMBC correlations that linked the drimane moiety to the hydroxyl-substituted quinone.

The stereochemistry of quinone **7** was next explored. The methyl group at δ_H 0.84 assigned to H-15 showed a strong NOE correlation to the methyl singlet at δ_H 0.87 assigned to H-14 and also to the methylene at H-11, thus suggesting that they were *syn*. An NOE from δ_H 1.21 (H-5) to δ_H 2.42 (H-9) revealed that these protons were also *syn*. The same NOE correlations had previously been noted for **17**,[10] and confirmed that **7** and **17** possessed the same relative stereochemistry. However, the two compounds had opposite $[\alpha]_D$ values in $CHCl_3$, +61 for **7** (*c* 0.14) and −86 for **17** (*c* 0.13), which suggested that they differed in absolute configuration. Two additional pieces of evidence support the absolute configuration shown for **7**. Firstly, an enantiospecific synthesis of isozonarone (**15**), with an $[\alpha]_D$ +89 in MeOH, has been completed by Seifert et al.[11] When the optical rotation of **7** was remeasured in MeOH, the value of +103 (*c* 0.14, MeOH) supported the absolute configuration as shown. Secondly, the metabolite dysienone (**16**), whose absolute configuration shown has been verified by temperature dependent NMR study of an MPA ester, has an $[\alpha]_D$ of −12.5 ($CHCl_3$),[9] opposite in sign to that of **7**. In view of its structural and stereochemical similarity to hyatellaquinone (**12**), compound **7** was named as isohyatellaquinone.

The quinone **8** provided a molecular formula of $C_{22}H_{28}O_4$ by high-resolution mass measurement. Comparison of spectroscopic data of quinone **8** with those of cyclospongiaquinone-2 (**5**)[12] revealed many similarities including a quaternary carbon at δ_C 101.5 that was suggestive of a spiro centre at C-9. However, the presence of both a vinyl methyl at δ_H 1.63 and a trisubstituted double bond (δ_C 129.6 (d), 130.3 (s); δ_H 5.69) suggested that **8** was the dehydro analogue of **5**, and so is structurally equivalent to the algal metabolite isochromazonarol (**18**).[13] Quinone **9** also showed a molecular formula of $C_{22}H_{28}O_4$ by high-resolution mass measurement, while comparison of the ^{13}C NMR and 2D NMR data ($CDCl_3$) of **8** and **9** revealed an identical carbon skeleton. The major difference in **9** was that the chemical shifts for the AB methylene protons assigned to H-11 were at δ_H 2.75 and 3.31 (1H each, d, J=17.3 Hz) in contrast to δ_H 2.88 and 3.03 (1H each, d, J=17.2 Hz) in **8**. Consequently quinones **8** and **9** differed only in stereochemistry at C-9. For **8**, the methyl group at δ_H 0.90 assigned to H-15 showed a strong NOE to the methyl singlet at δ_H 0.91 assigned to H-14, and also an NOE to the H-11 proton at δ_H 3.03, therefore, C-11 was *syn* to Me-15.

The methyl group at δ_H 1.63 (H-12) showed an NOE to the H-11 proton at δ_H 2.88. For **9**, an NOE from the methine at δ_H 1.36, assigned to H-5, to the equatorial methyl at δ_H 0.86 (H-13) and to both methylene protons at H-11 revealed that C-11 was *syn* to H-5 (Fig. 1). Since a number of proton signals were poorly resolved in $CDCl_3$, NOESY experiments were also run for **8** and **9** in C_6D_6, which gave better proton resolution, again with the same stereochemical conclusions. In $CDCl_3$ the H-5 signals for **8** and **9** appear at δ_H 1.82 and 1.36, respectively, whereas the axial Me-15 signals are at δ_H 0.90 and 1.12, respectively. In each case the more downfield proton signal results from deshielding by the C-9 oxygen substituent.[13]

We next compared the chemistry of *D. elegans* with the new species of *Dactylospongia* from which we had previously reported the cyclopropyl-containing dactylospongiaquinone (**1**) and other spongiaquinone-related quinones **2**–**5**.[2] Inspection of the ^1H NMR spectrum of a flash column fraction from the *Dactylospongia* sp. suggested the presence of quinone **7** in the extract. Isolation as described in Section 4 yielded a sample whose NMR spectra resembled those of **7**, but with an $[\alpha]_D$ value in $CHCl_3$ of −42.1 (*c* 0.07) compared to +61.6 (*c* 0.14) for the *D. elegans* sample. The *Dactylospongia* sp. sample was suggested to be *ent*-(**7**) rather than a diastereomer of **7** owing to the close matching of the NMR data.

An additional sesquiterpene component **13**, named as neomamanuthaquinone, was isolated from this second species of *Dactylospongia* and had a molecular formula of $C_{22}H_{30}O_4$ by high-resolution mass measurement of the $[M+Na]^+$ ion. The sesquiterpene portion revealed singlets for a *gem* dimethyl group at δ_H 0.93 and 0.97, a methyl singlet at δ_H 0.81, a methyl doublet at δ_H 0.76, plus the characteristic AB methylene system for the H-11 protons. In the ^{13}C NMR, alkene signals at δ_C 135.0 (s) and 131.5 (s) suggested a tetrasubstituted double bond. gHMBC correlations from the H-11 protons to the signal at δ_C 131.5 and from the *gem* dimethyl protons to the signal at δ_C 135.0 required that the double bond be placed between C-5 and C-10. The relative stereochemistry of metabolite **13** was confirmed by a selective NOE study. When the methyl singlet at δ_H 0.81 (Me-15) was irradiated, the methyl doublet for Me-12 as well as the H-11 protons was enhanced. Compound **13** has been reported as an isomerisation product of ilimaquinone[14] or of a ilimaquinone/5-*epi*-ilimaquinone mixture,[15] but without rigorous characterisation. The structural features are also present in the quinone compound **19**, obtained from rearrangement of isospongiaquinone (**20**) under acidic conditions followed by methylation,[16] and in the intermediate **21**, an unexpected reaction product isolated during the total synthesis of spongiaquinone.[6c] Both **19** and **21** showed comparable NMR data to **13** for the drimane component. Neomamanuthaquinone (**13**) showed $[\alpha]_D$ +3.3 (*c* 0.14, $CHCl_3$), compared to $[\alpha]_D$ +11 (*c* 0.09, $CHCl_3$) reported for the ilimaquinone rearrangement product,[14] and on methylation gave a dimethyl ether with NMR data identical with (**19**) and with an $[\alpha]_D$ of +14.6 (*c* 0.10, $CHCl_3$). Capon reported an $[\alpha]_D$ of +2.4 (*c* 0.9, $CHCl_3$) for (**19**),[16a] but did not record an $[\alpha]_D$ value for the sample of isospongiaquinone (**20**) from which it was derived.[17] The co-isolation of quinone **13** with (−)-spongiaquinone (**2**)[2] supports the argument that the compound should belong to the same enantiomeric series as (**2**) and (**20**) (but see below).

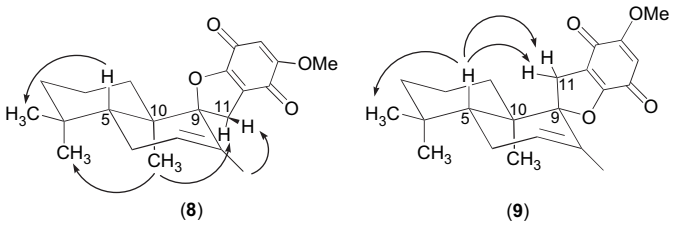

Figure 1. Selected NOE correlations observed for metabolites **8** and **9**.

Table 2
Cytotoxic activities of compounds **7**, *ent*-**7** and **8**–**13**

Compounds	IC_{50}[a,b] (μg/mL)	
	BC	NCI-H187
7	6.69	11.52
ent-**7**	Inactive	Inactive
8	Inactive	Inactive
9	7.38	12.40
10	2.61	8.78
11	1.50	3.37
12	4.45	10.90
13	8.42	Inactive

[a] IC_{50} at 20 μg/mL is regarded as inactive.
[b] The standard drug doxorubicin showed IC_{50} values of 0.29 and 0.06 μg/mL for breast cancer (BC) and small cell lung cancer (NCI-H187) cells, respectively.

In addition we identified the sesterterpene furospinosulin-1 (**22**),[18] often incorrectly named as furospinulosin-1 in the literature, as the major metabolite of the non-polar fraction of *D. elegans*. This metabolite has frequently been reported from quinone-containing sponges.[15,19]

Compounds **7**–**13** and *ent*-**7** were screened against breast cancer (BC) and small cell lung cancer (NCI-H187) cell lines, as shown in Table 2. For cytotoxic activity against the BC cell line, metabolites **10**, **11** and **12** were strongly active, whereas compounds **7**, **9** and **13** were moderately active. Only compound **11** was strongly active against the NCI-H187 cell line while compound **10** was moderately active and compounds **7**, **9** and **12** were weakly active. Metabolites *ent*-**7** and **8** were inactive against both cell lines while compound **13** was inactive to the NCI-H187 cell line. The exocyclic double bonds in **11** and **12**, as well as a 5,6-endocyclic double bond in **10**, seemed to contribute to high cytotoxicity against the BC cell line, while the exocyclic double bond in **11** may also explain the activity towards the NCI-H187 cell line.

2.2. Biosynthetic considerations

Figures 2 and 3 show suggested biosynthetic schemes towards the group of sesquiterpene quinones isolated in this study. The most interesting structures are the isomers **8** and **9**, since they may derive from a cationic intermediate such as **A** (Fig. 2). Their formation may be explained by double bond introduction giving **7**, then cyclisation (route a), or hydride migration followed by cyclisation giving a cyclic intermediate related to cyclospongiaquinone-2 (**5**), then double bond formation (route b). Either route is plausible. The formation of dictyoceratidaquinone (**6**) is explained by route (c) in which hydride migration, then the loss of a proton from a methyl adjacent to the cationic site ensures cyclopropyl ring formation. In Figure 3, initial cyclisation of the farnesyl precursor generates the enantiomeric cation **B**, which undergoes hydride and methyl migrations as shown to yield compounds **10**, **11** and **13** while loss of a proton generates *ent*-(**7**).

Examined together, the proposed biosynthetic routes provide an appropriate background for a discussion on absolute configuration. Both enantiomers of **7** were isolated during this study, although from different *Dactylospongia* sponges. The occurrence of antipodal sesquiterpenes, which is common in terrestrial natural products[20] is also encountered in the marine terpene literature. For example, the algae *Dictyopteris undulata* and *Dictyopteris zonarioides* produce isozonarol,[8] chromazonarol[21] and yahazunol[22] while sponges of the genus *Dysidea* contain *ent*-isozonarol,[9] *ent*-chromazonarol[23] and *ent*-yahazunol.[9] Enantiomers of the sesquiterpenes furodysinin and euryfuran have been reported from *Dysidea* sponges, but in each case the antipodal samples were collected from different geographic locations.[24,25] Curiously, the possibility of enantiomers is frequently ignored in marine stereochemical investigations that correlate new terpenes to known metabolites. Consequently such studies are

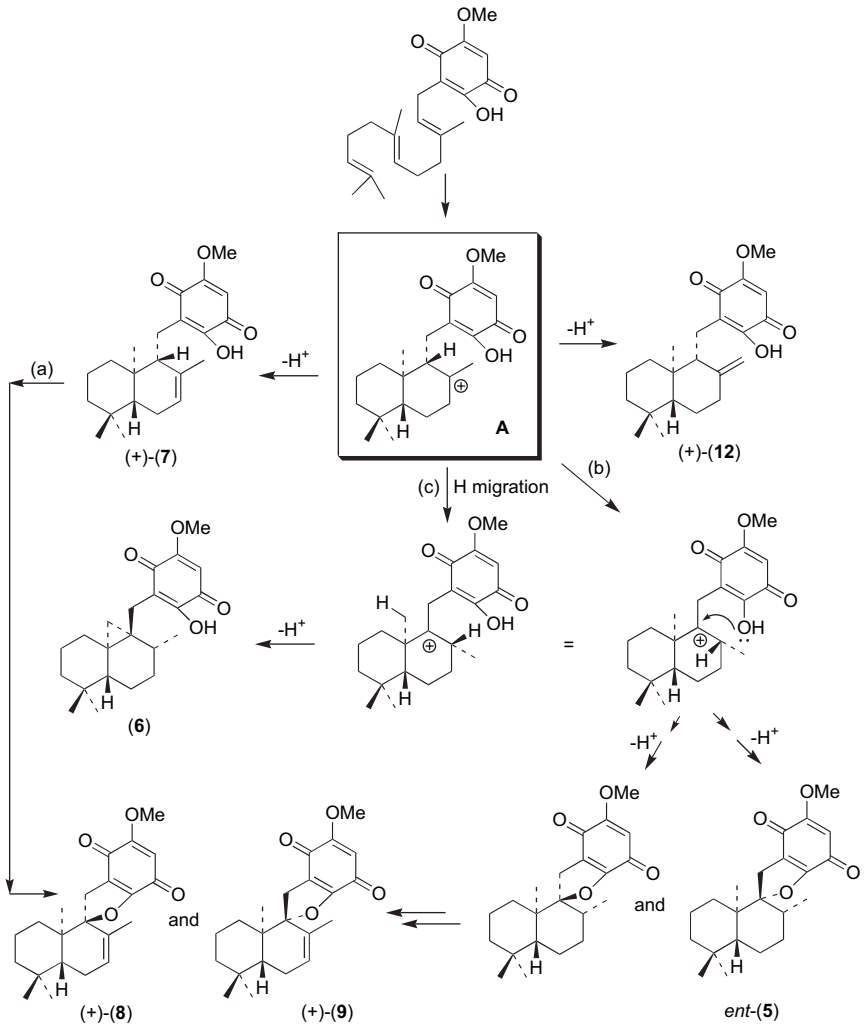

Figure 2. Suggested biosynthetic pathways leading to compounds **6**–**9**, **12** and related sesquiterpene quinones.

ambiguous unless the enantiomeric form of the known terpene is documented. We emphasise the importance of citing $[\alpha]_D$ values for known terpenes in cases where these metabolites are used as reference compounds in stereochemical investigations.[5b,16]

In our study, the well known metabolites (−)-ilimaquinone (**11**) and (+)-hyatellaquinone (**12**) were isolated from *D. elegans*. The absolute configurations of these two metabolites have been secured, by chemical correlation with aureol for **11**,[5b] and by total synthesis for **12**.[6b,c] The biosynthetic pathways leading to (−)-ilimaquinone (**11**) and (+)-hyatellaquinone (**12**) require cyclisation modes involving the antipodal cationic intermediates **B** (Fig. 3) and **A** (Fig. 2), respectively. Consequently in *D. elegans*, the isolation of these two compounds *from a single specimen* suggests that these alternative cyclisation modes can operate in parallel. In an earlier, and at the time unrecognised, report of antipodal sesquiterpene quinones from the same sponge specimen,[26] Capon described hyatellaquinone (**12**), characterised as its (+)-methyl ether (**23**),[6b,c] together with (−)-spongiaquinone (**2**) (whose configuration derives from cation **B** as shown in Fig. 3). Consequently, the commonly made assumption[24] that sesquiterpene quinones from the same sponge collection should necessarily belong to the same enantiomeric series is not supported by these results.

For marine sesquiterpene metabolites, it could well be that differences in absolute stereochemistry are determined by precursor binding preferences within the active site of a single cyclase enzyme.[27] Mechanistic studies using plant monoterpene cyclases (synthases) have revealed an interesting capacity to process chiral precursors. The plant *Salvia officinalis* contains a synthase enzyme that does not distinguish between enantiomeric intermediates formed by the folding of the achiral precursor since the two enantiomers are closely isosteric. As a result, this enzyme is capable of converting either 3*R* or 3*S* linalyl pyrophosphate (LPP) to chiral bornyl products.[28] Similarly, another synthase has been isolated from *S. officinalis*, which preferentially uses (3*S*)-LPP to form limonene, but can also process the 3*R* isomer, generating (*ent*)-limonene albeit at a reduced rate.[29] Detailed enzymological studies have given further insight into the capacity of plant systems to biosynthesise enantiomeric terpenes. In a pioneering study, fractionation of a cell-free extract from *S. officinalis* led to the purification of two enzymes producing antipodal pinenes.[20]

Consequently, the occurrence of antipodal terpenes in sponges may alternatively be explained by the presence of multiple synthase enzymes with each individual synthase showing some metabolic plasticity and so being capable of generating a range of different products, and presumably the capacity to alter stereochemical outcomes, depending on the nature of the substrate provided. In the terrestrial literature, there is now an increasing body of evidence that plants contain multiple terpene synthases, and that these enzymes are capable of aberrant biosynthetic processes.[30] The stereochemistry and mechanism of terpene cyclisations, and the underlying enzymology, are issues of considerable interest that warrant detailed investigation in marine

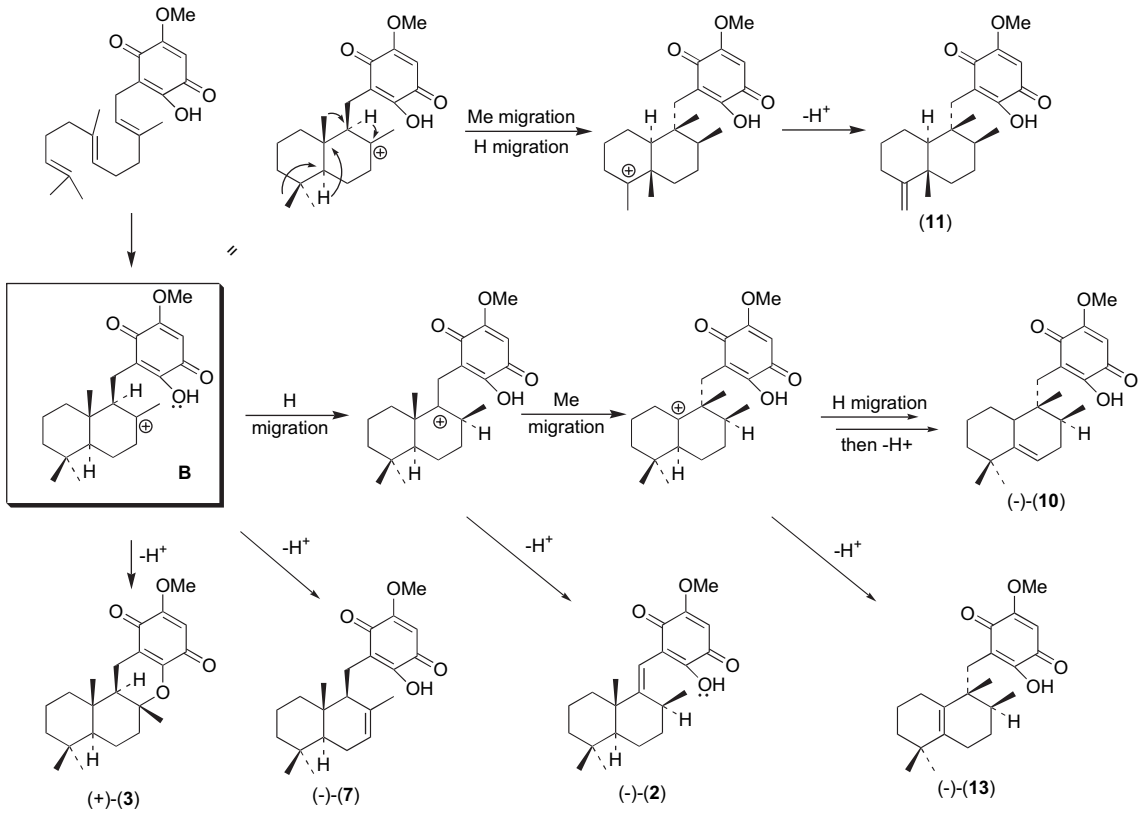

Figure 3. Suggested biosynthetic pathways leading to compounds *ent*-**7**, **10**, **11**, **13** and related sesquiterpene quinones.

sponges, given the bioactivity shown by many sponge terpene metabolites.

3. Conclusions

This study reported three new sesquiterpene quinones isohyatellaquinone (**7**), 7,8-dehydrocyclospongiaquinone-2 (**8**) and 9-*epi*-7,8-dehydrocyclospongiaquinone-2 (**9**) together with the known quinones dictyoceratidaquinone (**6**), mamanuthaquinone (**10**), ilimaquinone (**11**), hyatellaquinone (**12**), and the sesterterpene furospinosulin (**22**). A second species of *Dactylospongia* was found to contain *ent*-(**7**) together with the new quinone neomamanuthaquinone (**13**). The isolation of antipodal sesquiterpenes from closely related species has implications for the stereochemical evaluation of terpene metabolites and for the biosynthetic processes in marine sponges that involve terpene synthases.

4. Experimental

4.1. General experimental procedures

Optical rotations were obtained using a JASCO-P1010 polarimeter. 1D and 2D NMR spectra were acquired using Bruker AVANCE 400, Bruker DRX-500 or Bruker DMX-750 instruments. NMR spectra were obtained in deuterochloroform and deuterobenzene at room temperature, and were internally referenced to $CHCl_3$ at δ_H 7.24 or $CDCl_3$ at δ_C 77.0 and C_6H_6 at δ_H 7.15 or C_6D_6 at δ_C 128.0. Positive ion electrospray mass spectra (LRESMS) were determined using a Bruker Esquire HCT or Finnigan LC-Q instrument and HRESMS using a MicroTof Q instrument, each with a standard ESI source. Samples were introduced into the source using MeOH as solvent. Normal phase HPLC was carried out using a Waters 515 pump with a Waters 10 μ μPorasil 7.8×300 mm column and a Gilson 132 series RI detector with EtOAc/hexanes (3:7) as solvent. Supports for $AgNO_3$-impregnated chromatography were prepared by exposing the silica gel to an 8% solution of $AgNO_3$ in acetonitrile, and then drying the impregnated silica before use.

4.2. Biological material

A specimen of *D. elegans* (Thiele 1899) was collected from Coral Gardens dive site at the Inner Gneerings reef, a group of shoals near Mooloolaba (Australia), using SCUBA at a depth of 10–15 m on 20th January 2007. The sponge was dark-coloured and compressible, yet firm when cut. A voucher specimen (QM G324844) is lodged at the Queensland Museum. A specimen of another species of *Dactylospongia* is likely to be new to science given there are currently only two described species in this genus. It was collected from the Trench dive site at the Inner Gneerings reef at a depth of 10–15 m on 16th January 2006. 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 and a morphological description of the sponge are available from the authors. Samples were taken back to the laboratory where they were stored at −20 °C until extraction.

4.3. Extraction and isolation of quinones

The specimen of *D. elegans* (wet weight 31 g) was diced and extracted exhaustively with CH_2Cl_2/MeOH (1:1). The extract was removed, filtered through cotton and then evaporated under reduced pressure to give an aqueous residue, which was partitioned sequentially with EtOAc and *n*-BuOH. The EtOAc fraction was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure to give a brown extract (178 mg), which was subjected to Si flash

chromatography with gradient elution (hexanes→CH_2Cl_2→EtOAc→MeOH) to give 19 fractions coded NP1–NP13. The fractions NP2–4 that eluted from hexanes/CH_2Cl_2 [(4:1) to (1:1)] were combined and analysed by TLC and ^{1}H NMR yielding the known terpene furospinusolin (**22**).[18] Flash column fractions eluted using CH_2Cl_2/EtOAc [(4:1), (3:2) and (1:1)] all contained a mixture of quinone components. The CH_2Cl_2/EtOAc (4:1) fraction NP8 was rechromatographed on silica gel with gradient elution (CH_2Cl_2→EtOAc), then by silver nitrate-impregnated TLC ($AgNO_3$-TLC) using hexanes/EtOAc (9:1) as eluant yielding quinone **9**. The CH_2Cl_2/EtOAc (3:2) fraction NP9 was subjected to silver nitrate ($AgNO_3$) silica gel flash chromatography using gradient elution (hexanes→EtOAc). The components that eluted in hexanes/EtOAc (9:1) were further chromatographed on $AgNO_3$-TLC using hexanes/EtOAc (9:1; multiple development) to give mamanuthaquinone (**10**) and dictyoceratidaquinone (**6**). Components from the fractions that eluted in hexanes/EtOAc (8:2) were also rechromatographed using $AgNO_3$-TLC using hexanes/EtOAc (9:1) to provide the quinones **7** and **8**. The fractions of the $AgNO_3$–Si flash column that eluted in hexanes/EtOAc (1:1) and EtOAc (100%) gave ilimaquinone (**11**) by $AgNO_3$-TLC. The Si chromatography fraction NP11 that eluted with CH_2Cl_2/EtOAc (1:1) was also rechromatographed on $AgNO_3$-TLC yielding hyatellaquinone (**12**).

The other specimen of *Dactylospongia* (wet weight 163 g) was extracted as described previously.[2] The hexanes extract (960 mg) was subjected to gradient elution on silica gel flash chromatography (hexanes→CH_2Cl_2→MeOH). The fractions that eluted with hexanes/CH_2Cl_2 (1:1) were combined and further purified using semi-preparative NP-HPLC with hexanes/EtOAc (3:7) yielding quinone **13**. The flash column fractions that eluted with hexanes/CH_2Cl_2 (1:4) were also rechromatographed by $AgNO_3$-TLC using hexanes/EtOAc (5:1; multiple development) to give *ent*-(**7**).

4.3.1. *Dictyoceratidaquinone (**6**)*[3]

Compound **6** (0.28 mg) was obtained as a yellow amorphous solid. ^{1}H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 500 MHz)—see Table 1; HMBC ($CDCl_3$, 500 MHz) H-11 to C-9/C-15/C-1′/C-2′/C-6′, H-12 to C-7/C-8/C-9, H-13 to C-3/C-4/C-5/C-14, H-14 to C-3/C-4/C-5/C-13, H-15 to C-1/C-5/C-8/C-9/C-10/C-11, H-4′ to C-6′.

4.3.2. *Isohyatellaquinone (**7**)*

Compound **7** (2.70 mg) was obtained as a dark yellow amorphous solid. $[\alpha]_D$ +61.6 (*c* 0.14, $CHCl_3$), $[\alpha]_D$ +103.0 (*c* 0.14, MeOH); ^{1}H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 500 MHz)—see Table 1; ^{1}H NMR (C_6D_6, 500 MHz) δ_H 5.44 (1H, br s, H-7), 5.22 (1H, s, H-4′), 2.72 (3H, s, OMe), 2.67 (1H, dd, *J*=13.6, 8.4 Hz, H-11) and 2.53 (1H, dd, *J*=13.6, 3.5 Hz, H-11), 2.62 (1H, m, H-9), 2.00 and 1.42 (1H each, m, H-1), 1.92 and 1.86 (1H each, m, H-6), 1.75 (3H, br s, H-12), 1.53 and 1.44 (1H each, m, H-2), 1.36 (1H, m, H-3) and 1.14 (1H, ddd, *J*=13.2, 13.2, 3.4 Hz, H-3), 1.28 (1H, dd, *J*=11.9, 5.0 Hz, H-5), 0.94 (3H, s, H-15), 0.87 (3H, s, H-14) and 0.83 (3H, s, H-13); ^{13}C NMR (C_6D_6, 500 MHz) δ_C 182.4 (C, C-5′), 181.3 (C, C-2′), 161.4 (C, C-3′), 151.2 (C, C-6′), 135.0 (C, C-8), 123.1 (CH, C-7), 120.8 (C, C-1′), 101.9 (CH, C-4′), 55.5 (OMe-3′), 52.0 (CH, C-9), 50.4 (CH, C-5), 42.4 (CH_2, C-3), 39.4 (CH_2, C-1), 37.7 (C, C-10), 33.6 (CH_3, C-13), 33.2 (C, C-4), 24.1 (CH_2, C-6), 22.7 (CH_3, C-12), 22.3 (CH_3, C-14), 21.8 (CH_2, C-11), 19.4 (CH_2, C-2) and 13.8 (CH_3, C-15); HMBC ($CDCl_3$, 500 MHz) H-5 to C-4/C-6/C-9/C-10/C-13/C-14, H-9 to C-7/C-8/C-10, H-11 to C-8/C-9/C-10/C-1′/C-2′/C-6′, H-12 to C-7/C-8/C-9, H-13 to C-3/C-4/C-5/C-14, H-14 to C-3/C-4/C-5/C-13, H-15 to C-1/C-5/C-9/C-10, H-4′ to C-2′/C-3′/C-5′/C-6′, OMe-3′ to C-3′, 6′-OH to C-5′; HMBC (C_6D_6, 500 MHz) H-1 to C-3/C-9/C-15, H-5 to C-6/C-9/C-10/C-13/C-14/C-15, H-7 to C-5/C-6/C-9/C-12, H-11 to C-8/C-9/C-10/C-1′/C-2′/C-6′, H-12 to C-7/C-8/C-9, H-13 to C-3/C-4/C-5/C-14, H-14 to C-3/C-4/C-5/C-13, H-15 to C-1/C-5/C-9/C-10; HRESIMS *m*/*z* 381.2036, calcd for $C_{22}H_{30}NaO_4$ $[M+Na]^+$, Δ −0.6 mmu.

4.3.3. *ent-Isohyatellaquinone [ent-(**7**)]*

Compound **7** (0.7 mg) was obtained as a dark yellow amorphous solid. $[\alpha]_D$ −42.1 (*c* 0.07, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 7.37 (1H, s, OH), 5.81 (1H, s, H-4′), 5.37 (1H, br s, H-7), 3.84 (3H, s, OMe), 2.46 (2H, m, H-11), 2.42 (1H, m, H-9), 1.95 and 1.18 (1H each, m, H-1), 1.88 and 1.83 (1H each, m, H-6), 1.53 (1H, s, H-12), 1.50 and 1.40 (1H each, m, H-2), 1.40 and 1.10 (1H each, m, H-3), 1.25 (1H, m, H-5), 0.86 (3H, d, H-14), 0.83 (3H, s, H-13) and 0.83 (3H, s, H-15); ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C 182.5 (C, C-5′), 182.0 (C, C-2′), 161.0 (C, C-3′), 151.0 (C, C-6′), 134.8 (C, C-8), 122.8 (CH, C-7), 121.2 (C, C-1′), 102.0 (CH, C-4′), 56.7 (OMe-3′), 51.6 (CH, C-9), 50.1 (CH, C-5), 42.1 (CH_2, C-3), 39.0 (CH_2, C-1), 37.0 (C, C-10), 33.3 (CH_3, C-13), 33.0 (C, C-4), 23.7 (CH_2, C-6), 22.0 (CH_3, C-14), 22.0 (CH_3, C-12), 19.0 (CH_2, C-2), 21.4 (CH_2, C-11) and 13.5 (CH_3, C-15); HMBC ($CDCl_3$, 400 MHz) H-5 to C-4/C-6/C-10/C-13/C-14/C-15, H-9 to C-7/C-8/C-10, H-11 to C-8/C-9/C-1′/C-2′/C-6′, H-12 to C-7/C-8, H-13 to C-4/C-5/C-14, H-14 to C-4/C-5/C-13, H-15 to C-9/C-10, H-4′ to C-2′/C-3′/C-5′/C-6′, OMe-3′ to C-3′, 6′-OH to C-6′; HRESIMS *m*/*z* 359.2225, calcd for $C_{22}H_{31}O_4$ $[M+H]^+$, Δ +0.3 mmu.

4.3.4. *7,8-Dehydrocyclospongiaquinone-2 (**8**)*

Compound **8** (0.66 mg) was obtained as a yellow amorphous solid. $[\alpha]_D$ +38.0 (*c* 0.04, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 500 MHz)—see Table 1; ^{1}H NMR (C_6D_6, 500 MHz) δ_H 5.37 (1H, br s, H-7), 5.21 (1H, s, H-4′), 2.88 (1H, d, *J*=17.1 Hz, H-11) and 2.76 (1H, d, *J*=17.1 Hz, H-11), 2.81 (3H, s, OMe), 1.89 (1H, dd, *J*=12.1, 4.9 Hz, H-5), 1.81 and 1.63 (1H each, m, H-6), 1.46 (3H, s, H-12), 1.36 and 1.09 (1H each, m, H-1), 1.33 and 1.29 (1H each, m, H-2), 1.20 (1H, m, H-3) and 1.05 (1H, ddd, *J*=13.0, 13.0, 4.0 Hz, H-3), 0.74 (3H, s, H-14), 0.68 (3H, s, H-13), 0.61 (3H, s, H-15); ^{13}C NMR (C_6D_6, 500 MHz) δ_C 179.1 (C, C-5′), 177.7 (C, C-2′), 160.8 (C, C-3′), 159.5 (C, C-6′), 131.1 (C, C-8), 128.7 (CH, C-7), 118.9 (C, C-1′), 103.9 (CH, C-4′), 100.4 (C, C-9), 55.1 (OMe-3′), 42.1 (CH, C-5), 41.5 (CH_2, C-3), 41.0 (C, C-10), 32.7 (C, C-4), 32.3 (CH_3, C-13), 30.9 (CH_2, C-1), 28.9 (CH_2, C-11), 24.0 (CH_2, C-6), 21.7 (CH_3, C-14), 18.0 (CH_3, C-12), 17.9 (CH_2, C-2), 14.8 (CH_3, C-15); HMBC ($CDCl_3$, 500 MHz) H-1 to C-2/C-15, H-5 to C-4/C-6/C-9/C-10/C-13/C-14/C-15, H-11 to C-8/C-9/C-10/C-1′/C-2′/C-5′/C-6′, H-12 to C-7/C-8/C-9, H-13 to C-2/C-3/C-4/C-5/C-14, H-14 to C-3/C-4/C-5/C-13, H-15 to C-1/C-4/C-9/C-10, H-4′ to C-2′/C-3′/C-5′/C-6′, OMe-3′ to C-3′, 6′-OH to C-6′; HMBC (C_6D_6, 500 MHz) H-5 to C-4/C-6/C-9/C-10/C-13/C14/C-15, H-11 to C-9/C-10/C-1′/C-2′/C-3′/C-6′, H-12 to C-7/C-8/C-9, H-13 to C-3/C-4/C-5/C-14, H-14 to C-3/C-4/C-5/C-13, H-15 to C-1/C-5/C-9/C-10, H-4′ to C-2′/C-3′/C-5′/C-6′, OMe-3′ to C-3′, 6′-OH to C-6′; HRESIMS *m*/*z* 379.1885, calcd for $C_{22}H_{28}NaO_4$ $[M+Na]^+$, Δ −0.1 mmu.

4.3.5. *9-epi-7,8-Dehydrocyclospongiaquinone-2 (**9**)*

Compound **9** (0.78 mg) was obtained as an orange amorphous solid. $[\alpha]_D$ −10.0 (*c* 0.05, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 500 MHz)—see Table 1; ^{1}H NMR (C_6D_6, 500 MHz) δ_H 5.24 (1H, s, H-4′), 5.04 (1H, br s, H-7), 3.21 (1H, d, *J*=17.4 Hz, H-11) and 2.62 (1H each, d, *J*=17.4 Hz, H-11), 2.82 (3H, s, OMe), 1.67 (2H, m, H-6), 1.54 (3H, s, H-12), 1.34 and 1.20 (1H each, m, H-2), 1.33 and 1.10 (1H each, m, H-1), 1.20 and 0.83 (1H each, m, H-3), 1.14 (3H, s, H-15), 1.11 (1H, m, H-5), 0.75 (3H, s, H-14), 0.69 (3H, s, H-13); ^{13}C NMR (C_6D_6, 500 MHz) δ_C 178.8 (C, C-5′), 177.4 (C, C-2′), 160.8 (C, C-3′), 160.2 (C, C-6′), 136.3 (C, C-8), 122.1 (CH, C-7), 118.9 (C, C-1′), 104.0 (CH, C-4′), 101.3 (C, C-9), 55.2 (OMe-3′), 43.6 (CH, C-5), 41.8 (C, C-10), 41.4 (CH_2, C-3), 35.7 (C_2, C-11), 32.7 (C, C-4), 32.5 (CH_3, C-13), 30.8 (CH_2, C-1), 23.8 (CH_2, C-6), 21.5 (CH_3, C-14), 17.8 (CH_2, C-2), 16.9 (CH_3, C-12), 15.2 (CH_3, C-15); HMBC ($CDCl_3$, 500 MHz) H-1 to C-2/C-10/C-15, H-3 to C-1/C-4/C-5, H-5 to C-3/C-4/C-6/C-9/C-14/C-15, H-6 to C-7/C-8/C-10, H-11 to C-8/C-9/C-10/C-1′/C-2′/C-5′/C-6′, H-12 to C-7/C-8/C-9, H-13 to C-2/C-3/C-4/C-5/C-14, H-14 to C-3/C-4/C-5/C-13, H-15 to C-1/C-4/C-5/C-9/C-10, H-4′ to C-2′/C-3′/C-5′/C-6′, OMe-3′ to C-3′, 6′-OH to C-6′; HMBC (C_6D_6, 500 MHz) H-3 to C-1, H-5 to

C-6/C-9/C-10/C-13/C-14/C-15, H-6 to C-8, H-11 to C-8/C-9/C-10/C-1′/C-2′/C-5′/C-6′, H-12 to C-6/C-7/C-8/C-9, H-13 to C-3/C-4/C-5/C-10/C-14, H-14 to C-4/C-5/C-10/C-13, H-15 to C-1/C-5/C-9/C-10, H-4′ to C-2′/C-3′/C-5′/C-6′, OMe-3′ to C-3′/C-4′; HRESIMS *m*/*z* 379.1891, calcd for $C_{22}H_{28}NaO_4$ $[M+Na]^+$, Δ +0.6 mmu.

4.3.6. Mamanuthaquinone (**10**)[4]

Compound **10** (1.80 mg) was obtained as a yellow amorphous solid. $[\alpha]_D$ −110 (*c* 0.12, $CHCl_3$), lit.[4] $[\alpha]_{546}$ −31.0 (*c* 0.06, $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz) δ_H 7.42 (1H, s, OH), 5.82 (1H, s, H-4′), 5.37 (1H, br s, H-6), 3.84 (3H, s, OMe), 2.59 (1H, d, *J*=13.2 Hz, H-11) and 2.46 (1H, d, *J*=13.2 Hz, H-11), 2.09 (1H, m, H-10), 1.97 and 1.75 (1H each, m, H-7), 1.80 and 0.92 (1H each, m, H-1), 1.48 and 1.40 (1H each, m, H-2), 1.37 (1H, m, H-8), 1.35 and 1.13 (1H each, m, H-3), 1.01 (3H, s, H-13), 0.98 (3H, d, H-12), 0.94 (3H, s, H-14) and 0.73 (3H, s, H-15); ^{13}C NMR ($CDCl_3$, 500 MHz) δ_C 182.3 (C, C-5′), 182.0 (C, C-2′), 161.5 (C, C-3′), 152.8 (C, C-6′), 146.2 (C, C-5), 118.2 (CH, C-1′), 114.7 (C, C-6), 102.0 (CH, C-4′), 56.8 (OMe-3′), 41.6 (CH, C-10), 41.3 (CH_2, C-3), 40.7 (C, C-9), 36.4 (CH, C-8), 36.3 (C, C-4), 32.7 (CH_2, C-11), 31.4 (CH_2, C-7), 30.6 (CH_2, C-1), 29.7 (CH_3, C-13), 27.9 (CH_3, C-14), 22.7 (CH_2, C-2), 16.6 (CH_3, C-12) and 16.0 (CH_3, C-15); HMBC ($CDCl_3$, 500 MHz) H-6 to C-4/C-7/C-8/C-10, H-7 to C-6, H-8 to C-6, H-11 to C-1′/C-2′/C-6′, H-12 to C-7/C-8/C-9, H-13 to C-4/C-5/C-7/C-14, H-14 to C-4/C-5/C-7/C-13, H-15 to C-8/C-9/C-10, H-4′ to C-2′/C-3′/C-5′/C-6′, OMe-3′ to C-3′/C-4′, 6′-OH to C-5′/C-6′; LRESIMS *m*/*z* 381 $[M+Na]^+$.

4.3.7. Ilimaquinone (**11**)[5]

Compound **11** (5.39 mg) was obtained as a yellow amorphous solid. $[\alpha]_D$ −32.5 (*c* 0.12, $CHCl_3$), lit.[5] $[\alpha]_D$ −23.2 (*c* 1.12, $CHCl_3$); NMR data were all in accordance with literature values; LRESIMS *m*/*z* 381 $[M+Na]^+$.

4.3.8. Hyatellaquinone (**12**)[6]

Compound **12** (0.89 mg) was obtained as a yellow amorphous solid. $[\alpha]_D$ +37.4 (*c* 0.05, $CHCl_3$), lit.[6] $[\alpha]_D$ +15.6 (*c* 0.5, $CHCl_3$); NMR data were all in accordance with literature values; LRESIMS *m*/*z* 381 $[M+Na]^+$.

4.3.9. Neomamanuthaquinone (**13**)[14,15]

Compound **13** (1.40 mg) was obtained as a yellow amorphous solid. $[\alpha]_D$ +3.3 (*c* 0.14, $CHCl_3$), lit.[14] $[\alpha]_D$ +11 (*c* 0.09, $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 500 MHz)—see Table 1; HMBC ($CDCl_3$, 400 MHz); H-1 to C-10, H-6 to C-5, H-8 to C-12, H-11 to C-8/C-9/C-10/C-15/C-1′/C-2′/C-6′, H-12 to C-8/C-9, H-13 to C-3/C-4/C-5/C-14, H-14 to C-3/C-4/C-5/C-13, H-15 to C-9/C-10, H-4′ to C-2′/C-3′/C-5′/C-6′, OMe-3′ to C-3′, 6′-OH to C-1′/C-6′; HRESIMS *m*/*z* 381.2034, calcd for $C_{22}H_{30}NaO_4$ $[M+Na]^+$, Δ −1.1 mmu.

4.3.10. Neomamanuthaquinone methyl ether (**19**)[16]

A sample of **13** (0.9 mg, 0.0025 mmol) was subjected to methylation using MeI (0.23 μl, 0.0037 mmol, 1.5 equiv), K_2CO_3 (0.38 mg, 0.0027 mmol, 1.1 equiv) in anhydrous DMF (20 μl) and stirred 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 cotton wool 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 **19** (0.9 mg, 95%) as a yellow amorphous solid. $[\alpha]_D^{30}$ +14.6 (*c* 0.10, $CHCl_3$), lit.[16a] $[\alpha]_D$ +2.4 (*c* 0.9, $CHCl_3$); NMR data were in accordance with literature values;[16a] LRESIMS *m*/*z* 395 $[M+Na]^+$.

4.4. Cytotoxicity assays

The cytotoxicity assays against BC and NCI-H187 cells were performed employing a colourimetric method.[31] The standard drug doxorubicin exhibited IC_{50} values against these cell lines at 0.29 and 0.06 μg/mL, respectively.

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