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Dysidavarones A−D, New Sesquiterpene Quinones from the Marine Sponge *Dysidea avara*

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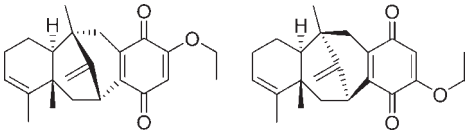
ABSTRACT



Dysidavarone A (**1**) Dysidavarone D (**4**)

Dysidavarones A−D (1−4), four new sesquiterpene quinones possessing the unprecedented "dysidavarane" carbon skeleton, were isolated from the South China Sea sponge *Dysidea avara*. The structures were established by spectroscopic methods, and the absolute configurations were determined using quantum mechanical calculation of the electronic circular dichroic (ECD) spectrum and exciton chirality CD method. Their cytotoxic activity against four human cancer cell lines and PTP1B inhibitory activity were also evaluated.

Marine sponges have proven to be an exceptionally valuable resource for bioactive natural product discovery. Metabolites isolated from marine sponges often possess unique structural features and show a broad range of potent biological activities.[1] Sesquiterpene quinones and hydroquinones represent a prominent class of mixed biogenesis metabolites that incorporate a bicyclic sesquiterpene moiety into a benzoquinone or a hydroquinone unit.[2] These metabolites were mainly obtained from marine sponges and their associated microorganisms, although some have been isolated from brown algae and other marine organisms.[2] Avarone and avarol, the most well-known members of this family, showed potent anti-HIV activity.[3] Many sesquiterpene quinones and hydroquinones also exhibited antibacterial,[4] antifungal,[5] antitumor,[6] anti-inflammatory,[7]

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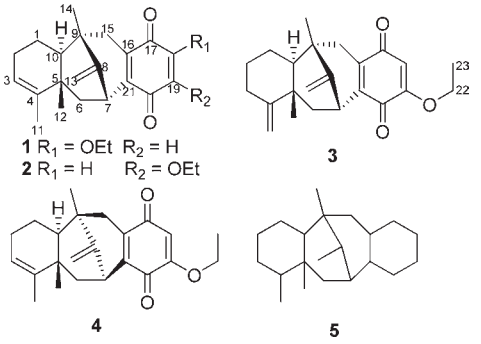
and antioxidant activities.[8] Their structural diversity, differences in biosynthetic pathways, and pharmaceutical potential have attracted continuing interest from both chemists and biologists. Many bioactive sesquiterpene quinones and hyroquinones have been discovered and synthesized.[9]

As part of our ongoing bioactive marine natural product discovery program,[10] we focused our efforts on the cytotoxic extracts of sponge *Dysidea avara*, collected off the Xisha islands. Cytotoxicity-guided fractionation yielded four sesquiterpene quinones, dysidavarones A−D (**1**−**4**), possessing the unprecedented dysidavarane (**5**) carbon skeleton. Details of the isolation, structure elucidation, plausible biogenetic pathways, and biological activity of the four metabolites are presented herein.

The EtOH extract of *D. avara* was partitioned between 90% MeOH and *n*-hexane, and the *n*-hexane-soluble portion was successively fractionated by VLC on Silica gel followed by size-exclusion chromatography on Sephadex LH-20 and purified on reversed-phase HPLC to yield dysidavarones A−D (**1**−**4**).



Dysidavarone A (**1**) was obtained as a yellowish oil and determined to have a molecular formula of $C_{23}H_{28}O_3$ by the HRESIMS ion peak at *m*/*z* 375.1937 $[M + Na]^+$, implying 10 degrees of unsaturation. The ^{1}H NMR and HSQC data of **1** revealed the presence of three aliphatic methyls, an olefinic methyl, five methylenes (one oxygenated), an exomethylene, two aliphatic methines, an olefinic methine, and an isolated quinone methine (Table 1). Based on the COSY data, these subunits were assembled into three partial structures: C-10−C-3, C-6−C-7, and an *O*-ethyl group (Figure 1). The elongation of the first partial structure from C-3 to C-11 was supported by a small allylic coupling constant (1.0 Hz) between H_3-11 and H-3. HMBC correlations from H_3-12 to C-4, C-5, C-6, and C-10 established the connectivity of C-4, C-10, and C-6 *via* a quaternary carbon C-5. Meanwhile the aliphatic methyl H_3-14 showed HMBC correlations with C-8, C-9, C-10, and C-15, which assigned the methyl group at C-9. In the low field, the singlet quinone proton H-19 showed HMBC correlations with C-17, C-18, C-20, and C-21, suggesting the presence of a benzoquinone unit confirmed by the carbon chemical shifts (Table 1). Moreover, the HMBC correlations from both H-15a and H-15b to C-16, C-17, and C-21, from both H-6a and H-6b to C-21, and from H-7 to C-16, C-20, and C-21 indicated the connectivity of C-15−C-16 and C-7−C-21. Additionally, the HMBC correlations from both H-13a and H-13b to C-7, C-8, and C-9 assigned a carbon bridge (C-8) between C-7 and C-9. Thus, the unusual tetracyclo[7.7.1.$0^{2,7}$.$0^{10,15}$]heptadecane core of **1** was finally revealed. The location of the *O*-ethyl group at C-18 was determined by the HMBC correlation from the oxymethylene H_2-22 to C-18 combined with the NOESY correlation of H_2-22/H-19.

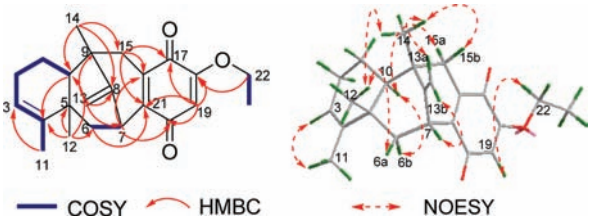


Figure 1. ^{1}H−^{1}H COSY, HMBC, and NOESY correlations of **1**.

The relative configuration of **1** was established by NOESY experiment. The NOESY correlations of H_3-12/H-6a and H-10/H-6b revealed the *trans*-fusion of the bicyclic system as supported by the chemical shift of the methyl CH_3-12 (δ_C 20.4).[6a] The β-orientation of the carbon bridge C-7−C-8−C-9 was assigned by the NOESY correlation of H_3-12/H_3-14, which was confirmed by *J*-based configuration analysis (Figure 2). The methine proton H-7 was assigned *cis* to H-6a and *gauche* to H-6b, according to the magnitude of the coupling constants provided by the ^{1}H NMR spectrum.

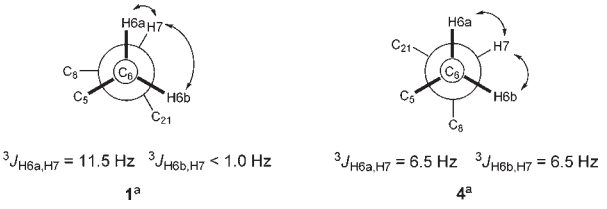


Figure 2. *J*-Based configuration analyses for **1** and **4**. [a]Rotamer designation according to Murata.[11]

The quantum chemical ECD calculation method[12] was used to further establish the absolute configuration of dysidavarone A (**1**). The preliminary conformational distribution search was performed by Syby18.0 software using the Tripos force field overlaid with key correlations observed in the NOESY spectrum. The corresponding

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Table 1. ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) Data of **1** and **4** in $CDCl_3$ (J in Hz)

	1		**4**	
no.	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1a	1.67, m	20.5	1.72, d, 8.0	21.6
1b	1.57, m		1.52, m	
2a	2.00, m	26.5	2.08, m	25.8
2b	1.86, m		1.98, m	
3	5.08, d, 1.0	119.5	5.31, d, 1.0	121.8
4		142.7		141.0
5		37.2		38.0
6a	2.16, dd, 14, 11.5	42.0	2.34, dd, 6.5, 3.0	41.2
6b	1.50, brd, 14		0.95, d, 6.5	
7	3.71, brd, 11.5	37.7	2.93, dt, 6.5, 1.5	48.8
8		152.6		153.1
9		37.9		45.5
10	1.67, m	47.3	2.11, m	45.1
11	1.52, 3H, d, 1.0	18.0	1.61, 3H, d, 1.0	18.5
12	1.04, 3H, s	20.4	0.81, 3H, s	17.0
13a	4.90, s	105.6	5.07, 1H, s	108.4
13b	4.84, s		4.87, 1H, s	
14	1.24, 3H, s	19.8	1.20, 3H, s	26.6
15a	2.78, d, 19.0	43.0	2.90, d, 18.5	42.5
15b	2.00, d, 19.0		2.42, d, 18.5, 2.0	
16		139.3		147.0
17		182.8		186.8
18		157.8	5.78, s	107.4
19	5.83, s	107.6		158.8
20		186.6		180.4
21		149.1		150.4
22	3.98, 2H, q, 7.0	65.0	3.99, 2H, q, 7.0	65.3
23	1.48, 3H, t, 7.0	13.9	1.47, 3H, t, 7.0	13.9

minimum geometries were further fully optimized by using DFT at the B3LYP/6-31G(d) level as implemented in the Gaussian 09 program package. The stable conformers obtained were submitted to ECD calculation by the TDDFT [B3LYP/6-31++G(2d,3p)] method. The overall predicted ECD spectrum of **1** was subsequently compared with the experimental one, which revealed a good agreement between the calculated and the measured ECD curves (Figure 3). Thus, the stereostructure of **1** was unambiguously established as 5*S*, 7*R*, 9*S*, 10*R*.

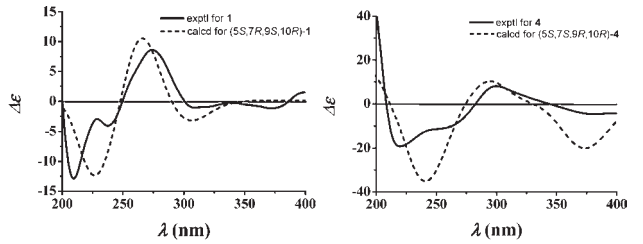

Figure 3. Experimental and calculated ECD spectra for **1** and **4**.

The CD spectrum of **1** showed a positive bisignate Cotton effect [λ 238 ($\Delta\varepsilon$ −4.16), 274 (+8.47)]. The latter was assigned to exciton coupling between the $\pi-\pi^*$ transitions of the two chromophores: the double bond C-8−C-13 and the benzoquinone unit. Application of the Harada−Nakanishi nonempirical rule for exciton chirality CD determined the positive bisignate Cotton effect for **1** (Figure 4).[13] The chirality of **1**, therefore, corresponds to 5*S*, 7*R*, 9*S*, 10*R*.

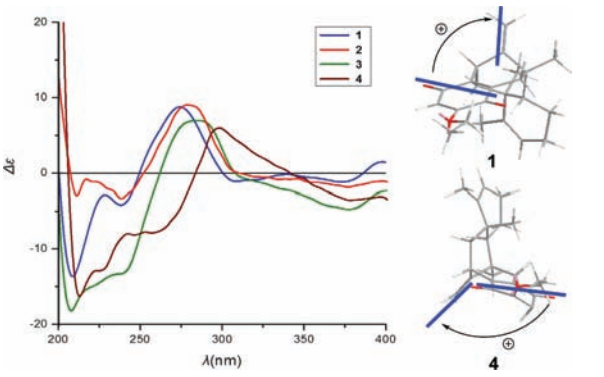

Figure 4. Experimental CD spectra of **1**−**4**. Stereoviews of **1** and **4**. Bold lines denote the electric dipole of the chromophores for **1** and **4**.

Dysidavarone B (**2**) was obtained as a pale yellow oil that gave an $[M + Na]^+$ ion peak at m/z 375.1937 in HRESIMS appropriate for the molecular formula of $C_{23}H_{28}O_3$. The ^{1}H and ^{13}C NMR data of **2** were similar to those of **1**, except for the six carbon signals in the benzoquinone unit (Supporting Information). The HMBC correlation between H_2-22 and C-19 and the NOESY correlation of H_2-22/H-18 placed the *O*-ethyl group at C-19, rather than C-18 as in **1**. Detailed analysis of the NOESY spectrum of **2** revealed that its relative configuration was consistent with that of **1** (Supporting Information).

Dysidavarone C (**3**) was assigned the same molecular formula $C_{23}H_{28}O_3$ as **1** and **2** by HRESIMS (m/z 375.1939 $[M + Na]^+$). The ^{1}H and ^{13}C NMR data of **3** corresponded closely to those of **2**, except for three subunits, an exomethylene CH_2-11 (δ_H 4.54, 4.01/δ_C 106.0), an sp^3 methylene CH_2-3 (δ_H 2.20, 2.06/δ_C 33.0), and an sp^2 quaternary carbon C-4 (δ_C 158.2). Detailed 2D NMR analysis revealed that the $\Delta^{3,4}$ double bond in **2** was transformed to an exocyclic double bond at C-4 in **3**. The NOESY correlations of H_3-12/H-6a, H-10/H-6b, and H_3-12/H_3-14 indicated that the relative configuration of **3** was consistent with that of **1** (Supporting Information).

The absolute configurations of **2** and **3** were also determined as 5*S*, 7*R*, 9*S*, 10*R* on account of the similar Cotton effects in the CD spectra of **2** and **3** to that of **1** (Figure 4 and Supporting Information).

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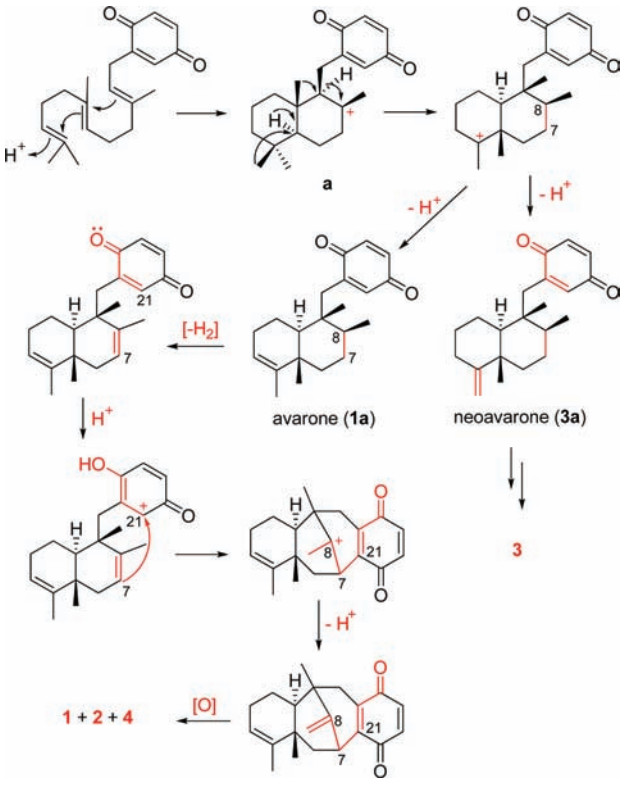
Scheme 1. Hypothetical Biogenetic Pathways for **1**−**4**



The HRESIMS of dysidavarone D (**4**) exhibited an $[M + Na]^+$ peak at m/z 375.1934, indicating the molecular formula of $C_{23}H_{28}O_3$ and 10 degrees of unsaturation. The ^1H and ^{13}C NMR data of **4** were similar to those of **2**, except for an sp^2 quaternary carbon at δ_C 147.0 (C-16), an sp^3 quaternary carbon at δ_C 45.5 (C-9), a methine at δ_H 2.93/δ_C 48.8 (CH-7), and two aliphatic methyls at δ_H/δ_C 0.81/17.0 (CH_3-12) and 1.20/26.6 (CH_3-14). In the HMBC spectrum, correlations from H_3-12 to C-4, C-5, C-6, and C-10 placed the methyl CH_3-12 at C-5, whereas the correlations from H_3-14 to C-8, C-9, C-10, and C-15 located the methyl at C-9. The placement of the methine CH-7 and the sp^2 quaternary carbon C-16 was determined by the HMBC correlations from H-7 to C-5, C-9, C-16, and C-21 (Supporting Information). Thus, the planar structure of **4** was determined unambiguously, which was intriguingly identical with that of **2**.

The NOESY correlations of H_3-12/H-15a and H_3-14/H-15b suggested the α-orientation of the carbon bridge C-7−C-8−C-9 in **4** (Supporting Information), which was confirmed by the *J*-based configuration analysis (Figure 2). Both diastereotopic methylene protons H-6a and H-6b showed the same coupling constant (6.5 Hz) with H-7, placing H-7 in the middle of the two protons. The absolute configuration of **4** was assigned as 5*S*, 7*S*, 9*R*, 10*R* by the quantum mechanical calculation of the ECD spectrum (Figure 3) and confirmed by the exciton chirality CD analysis (Figure 4 and Supporting Information).

Dysidavarones A−D (**1**−**4**) are the first natural products possessing the unprecedented dysidavarane (**5**) carbon skeleton. Biogenetically, the four new sesquiterpene quinones (**1**−**4**) might be generated from avarone and neoavarone as shown in Scheme 1.

All sesquiterpene quinone structures from the *Dysidea* species are believed to come from the farnesyl precursor *via* cationic cyclization and rearrangements.[3,4] The cyclization of the farnesyl precursor occurs by an initial electrophilic attack at the head position, which initiates a concerted process leading to a bicyclic carbocation intermediate (**a**), which converts to a 4,9-friedodrimane structure, avarone (**1a**) and neoavarone (**3a**).[3c] After double bond formation between C-7 and C-8, the intramolecular nucleophilic addition in avarone results in the formation of a new carbon bond C-7−C-21, establishing the unprecedented dysidavarane carbon skeleton and finally producing dysidavarones A−D (**1**−**4**).

Dysidavarones A (**1**) and D (**4**) were evaluated for their cytotoxicity against four human cancer cell lines, cervix (HeLa), lung (A549), breast (MDA231), and hepatoma (QGY7703), by an MTT method, using camptothecin as a positive control. Dysidavarone A (**1**) showed a growth inhibitory effect against HeLa cells with an IC_{50} value of 39.9 μM, and dysidavarone D (**4**) showed inhibitory effects against the four cell lines with IC_{50} values of 28.8, 21.4, 11.6, and 28.1 μM, respectively. In addition, dysidavarones A (**1**) and D (**4**) also showed inhibitory activity on protein tyrosine phosphatase 1B (PTP1B)[14] with IC_{50} values of 9.98 and 21.6 μM, respectively, using oleanolic acid as a positive control with an IC_{50} value of 1.90 μM.

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Supporting Information Available. Experimental procedure; ^1H,^1H−COSY, HMBC, NOESY correlations of **2**, **3**, and **4**; HRESIMS, 1D NMR, 2D NMR, and CD spectra of **1**−**4**. This material is available free of charge via the Internet at http://pubs.acs.org.

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