



## New Sesquiterpene Hydroquinones from Marine Brown Alga *Dictyopteris undulata*

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### Abstract

One new sesquiterpene hydroquinone, together with four known sesquiterpene hydroquinones and one hydrocarbon have been isolated from the bioassay-guided fractionation of the  $\text{CH}_2\text{Cl}_2$  extract of the marine brown alga *Dictyopteris undulata* (*zonarioides*). The structural elucidation is based on the analysis of spectroscopic data. This is the first report of isolation of 6 from this alga.

**Keywords:** *Dictyopteris undulata*, *Dictyotaceae*, sesquiterpene hydroquinone.

### Introduction

Brown algae belonging to the family Dictyotaceae have given rise to a great number of secondary metabolites possessing antibiotic, antifungal, antiviral, cytotoxic and antitumor activities<sup>1-5</sup>. Marine brown algae of the genus *Dictyopteris* have been reported to contain sesquiterpenoids and  $\text{C}_{11}$  hydrocarbons<sup>6-18</sup>.

In our search of antimycobacterial marine natural products we have found that the dichloromethane extract of *Dictyopteris undulata* possesses antimycobacterial activity. We report herein the isolation and structural elucidation of one new sesquiterpene hydroquinone 1 together with four known compounds<sup>2-6</sup>.

### Material and Methods

**Plant Material:** The marine brown alga *D. undulata* was collected at the Catalina Island, California (USA) in August 1997. The alga was frozen shortly after collection and kept frozen until the workup.

**Extraction and Isolation:** The dry marine brown alga *D. undulata* was exhaustively extracted with  $\text{CH}_2\text{Cl}_2$  at room temperature and the solvent was removed in vacuo at 30°C to yield a dark green residue (6.22 g). The crude residue showed antimycobacterial activity against *M. marinum* at a concentration of 0.2 µg/ml. The active crude residue (6.20g) was vacuum flash chromatographed over silica gel (400 g), eluting with gradient of increasing polarity of ethyl acetate in TMP to 10% MeOH in EtOAc yielding twelve fractions. Fraction 3 (TMP: 10%EtOAc, 510 mg) and fraction 11 (EtOAc: 5%MeOH, 431 mg) showed activity against *Mycobacterium marinum*. Fraction 3 was purified by chromatography on a column of silica gel with stepwise increases of EtOAc content (1-20%) in hexane. The fraction eluted with 4%, 6%, 10%, 12%, 14% EtOAc in hexane gave compounds 4, 6, 2, 3, and 1,

respectively. Fraction 11 was subjected to silica gel column chromatography and compound 5 was eluted with 50% ethyl acetate in iso-octane (2,2,4-Trimethylpentane or TMP), which was then crystallized in EtOAc.

**General experimental procedures:** Column chromatography was performed with Si gel (230-300 mesh), purchased from Aldrich. IR spectra were recorded as KBr disks on a Nicolet magna 550FTIR spectrophotometer. <sup>1</sup>H and <sup>13</sup>C NMR spectra were recorded at ambient temperature on a Bruker ARX-300 FT-NMR spectrometer operating at 300 MHz. Chemical shifts were recorded as δ values in parts per million (ppm), and were indirectly referenced to tetramethylsilane (TMS) via the solvent signal (e.g. 7.26 for <sup>1</sup>H, 77.0 for <sup>13</sup>C in  $\text{CDCl}_3$ ). Low resonance EI-MS was obtained on Hewlett Packard series II 5890 GC-MS. TLC was carried out with glass precoated silica gel GF<sub>254</sub> plates. Spots were visualized under UV light and by spraying with 50% aqueous sulphuric acid followed by heating.

### Results and Discussion

The  $\text{CH}_2\text{Cl}_2$  crude extract of *dictyopteris undulate* showed in vitro antimycobacterial activity against *mycobacterium marinum* and was subjected to activity-guided fractionation using vacuum flash chromatography followed by silica gel chromatography and Prep-TLC to yield compounds<sup>1-6</sup>.

Compound 1 was obtained as as colored gum and showed absorption bands for hydroxy ( $3400\text{ cm}^{-1}$ ) and ether ( $1254$  and  $1050\text{ cm}^{-1}$ ) functional groups and an exocyclic double bond ( $1640\text{ cm}^{-1}$ ) in the IR spectrum. The low resonance MS of 1 gave a molecular ion peak at  $m/z$  328  $[\text{M}]^+$ , and the molecular formula was established as  $\text{C}_{22}\text{H}_{32}\text{O}_2$ . The <sup>1</sup>H NMR spectrum in  $\text{CDCl}_3$  showed three methyl singlets at δ 0.83 ( $\text{CH}_3$ -13), 0.85 ( $\text{CH}_3$ -14), and 0.91 ( $\text{CH}_3$ -15), one methyl singlet attributed to a methoxy group at δ 3.51 ( $\text{CH}_3\text{O}$ ), two exocyclic methylene

proton singlets at  $\delta$  4.70 and 4.83 (H-12), and an hydroxy proton singlet at  $\delta$  4.60 (OH-5'), two doublets and one singlet for aromatic protons at  $\delta$  6.63,  $\delta$  6.52 and 6.61 respectively, as well as signals with complex coupling patterns attributed to methylene and methine protons. The  $^{13}\text{C}$  NMR spectrum showed 22 carbon signals including six aromatic carbons. The DEPT spectrum indicated the presence of four methyls (one methoxy), seven methylenes (one exocyclic double bond), five methines, and six quaternary carbons. The signal at  $\delta$  107.6 in the  $^{13}\text{C}$  NMR was characteristic of an exocyclic double bond. These data suggested that compound 1 was a sesquiterpene hydroquinone. On the basis of spectral data structure 1 was proposed for this compound, which was further confirmed by the spectral data of closely related compound 2<sup>6</sup>. The NMR spectra of 1 were similar to those of 2 except that the hydroxyl signal of 2 was replaced by methoxy signal at  $\delta$  3.51 and  $\delta$  5.09. The remaining  $^1\text{H}$  and  $^{13}\text{C}$ -NMR absorptions are consistent with the structural feature of zonarol 2<sup>6</sup>. A small amount was extracted with various solvents to confirm presence of methoxy zonarol 1 in alga rather than artifact of extraction, fractionation techniques.

## Conclusion

On the basis of spectroscopic data compound 1 was identified as a new sesquiterpene hydroquinone and five known compounds were identified as zonarol 2<sup>6</sup>, isozonarol 3<sup>6</sup>, Zonarone 4<sup>7</sup>, Yahazunol 5<sup>9</sup>, and a hydrocarbon neophytadiene 6<sup>19</sup> by comparing their physical and spectral data with those reported in the literature. Neophytadiene has been isolated for the first time from this alga. It is common that the same taxonomic species distributed in different regions contain different chemical constituents. The zonarol 2 and Yahazunol 5 were further confirmed by the spectral data of their diacetates 2a and 5a, respectively.

**Compound 1: IR( $\text{cm}^{-1}$ ):** 3400 (w), 2980(s), 2860(m), 2830(m), 1640 (w), 1595(w), 1495(s), 1455(m), 1380(m), 1315 (w), 1254 (w), 1050(m).

**LR EI-MS:** 55, 69, 81, 95, 109, 123, 135, 161, 178, 191, 207, 181, 315, 328[M<sup>+</sup>]. C<sub>22</sub>H<sub>32</sub>O<sub>2</sub>.

**Compound 2: FAB MS  $m/z$  :** 43(29), 48(33), 53(22), 55(60), 67(27), 69(45), 77(22), 79(26), 81(34), 83(21), 91(30), 95(38), 123(100), 109(27), 107(23), 137(32), 161(16), 163(13), 178(13), 191(53), 192(8), 314( 33, M<sup>+</sup>), C<sub>21</sub>H<sub>30</sub>O<sub>2</sub>.

**IR( $\text{cm}^{-1}$ ):** 3595 (w), 3355(w), 2925(s), 2860(m), 1640 (w), 1595(w), 1495(s), 1455(m), 1380(m), 1315 (w), 1285 (w), 1140(m), 1070 (w).

**Neophytadiene 4: LR EI-MS  $m/z$ :** 67 (100%), 69 (17%), 77 (17%), 79 (37%), 81 (50%), 82 (16%), 95 (49%), 109 (15%), 123 (20%), 278(1% M<sup>+</sup>). C<sub>20</sub>H<sub>38</sub>.

**Acetylation of compound 2 :** A solution of 20 mg of 2 in 1.0 ml pyridine was treated with 2 ml acetic anhydride and the reaction mixture left at room temperature for 24 hr. Dilution with water and extraction with ethylacetate yielded a diacetate 2a (15.2 mg).

**Acetylation of compound 5 :** A solution of 5.0 mg of 5 in 1.0 ml pyridine was treated with 2 ml acetic anhydride and the reaction mixture left at room temperature for 22 hr. Dilution with water and extraction with ethylacetate yielded a diacetate 5a (4.8 mg).

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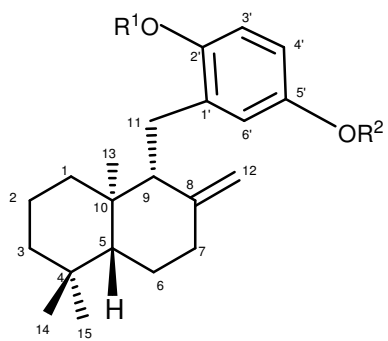
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**Table-1**  
**<sup>1</sup>H NMR and <sup>13</sup>C NMR data for Compound 1 and 2 (300 MHz in CDCl<sub>3</sub>)**

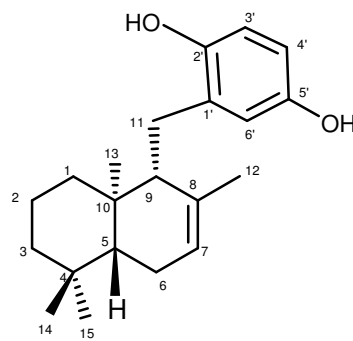
No.	1		2	
	$\delta_C$	$\delta_H$	$\delta_C$	$\delta_H$
1	39.10, t	1.19 m, 1.76 m	39.14, t	1.18 m, 1.75 m
2	19.39, t	1.54 m	42.09, t	1.22 m, 1.40 m
3	42.50, t	1.23 m, 1.41 m	42.09, t	1.22 m, 1.40 m
4	33.61, s	-----	33.62, s	-----
5	55.63, d	1.04 m	55.62, d	1.18 m
6	24.36, t	1.56 m	24.36, t	1.36 m
7	38.18, t	2.72 d (3)	38.18, t	2.05 d (9)
	-----	2.39 dd (15, 3)	-----	2.41 (12)
8	148.71, s	-----	148.71, s	-----
9	56.02, d	2.18 m	56.01, d	1.75 m
10	40.13, s	-----	40.14, s	-----
11	24.78, t	2.72 d (3)	23.59, t	2.72 d (6)
12	107.60, t	4.70 s, 4.83 s	107.61, t	4.70 s, 4.83 s
13	14.49, q	0.83 s	14.50, q	0.83 s
14	21.72, q	0.85 s	21.72, q	0.85 s
15	33.61, q	0.91 s	33.62, q	0.91 s
1'	130.10, s		129.76, s	-----
2'	149.10, s		147.50, s	-----
3'	115.79, d	6.63 d (6)	116.51, d	6.63 d (3)
4'	112. 87, d	6.52 d (3)	112.88, d	6.53 d (3)
5'	148.90, s	-----	147.50, s	
6'	116.51, d	6.61 s	115.80, d	6.61 s
OH	-----	4.60 brs	4.36 s	
OH	-----	-----	-----	4.52 s
OCH <sub>3</sub>	50.90, s	3.51 s	-----	-----

**Table-2**  
<sup>1</sup>H NMR and <sup>13</sup>C NMR data for Compound **2a** and **5a** ( 300 MHz in CDCl<sub>3</sub>)

No.	<b>2a</b>		<b>5a</b>	
	$\delta_c$	$\delta_H$	$\delta_c$	$\delta_H$
1	39.89, t	1.12 d (6), 1.04 m	40.1, t	1.85 m
2	19.36, t	1.43 m	18.5, t	1.51 m
3	42.07, t	1.22 m, 1.18 m	41.7, t	1.15, m
4	38.01, s	-----	33.3, s	-----
5	55.48, d	1.19 m	56.0, d	1.11 m
6	24.27, t	1.37 m	20.3, t	1.64 m
7	39.29, t	2.37 d (9)	44.1, t	1.89 m
8	148.01, s	-----	73.8, s	-----
9	55.60, d	2.07 m	62.3, d	1.56 m
10	39.89	-----	39.2, s	-----
11	23.83, t	2.75 d (6)	24.6, t	2.62 dd (17.1, 2.6)
12	108.53, t	4.52 s, 4.80 s	24.3, q	1.26 s
13	14.52, q	0.80 s	15.3, q	0.90 s
14	20.97, q	0.85 s	21.5, q	0.81 s
15	33.60, q	0.91 s	33.4, q	0.88 s
1'	135.66, s	-----	137.8, s	-----
2'	146.42, s	-----	145.6, s	-----
3'	122.44, d	6.90 d (3)	123.4, d	6.99 d (8.7)
4'	119.37, d	6.99 d (3)	123.0, d	6.90 dd (8.7, 2.6)
5'	147.76, s		148.1, s	
6'	122.67, d	6.93 s	119.4, d	7.13 d (2.6)
Acetate				
-CH <sub>3</sub>	21.17	2.30 s	21.2, q	2.35 s
	21.73	2.35 s	21.1, q	2.30 s
Acetate				
C=O	169.31	-----	169.4, s	-----
	169.31	-----	169.6, s	-----

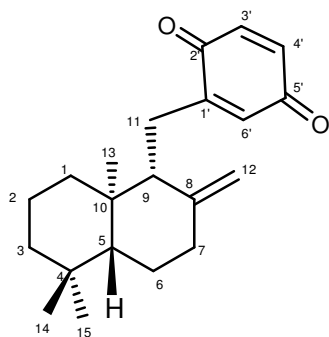


**1**  $R^1 = CH_3, R^2 = H$

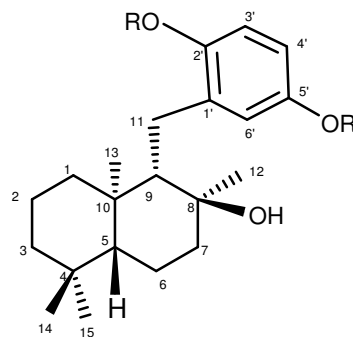


**3**

**2**  $R^1 = R^2 = H$   
**2a**  $R^1 = R^2 = COCH_3$

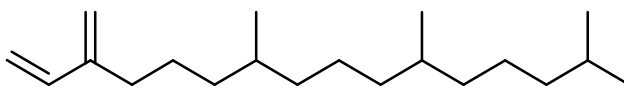


**4**



**5**  $R=H$

**5a**  $R=COCH_3$



**6**