

Meroditerpenoids from a Formosan Soft Coral *Nephthea chabrolii*Jui-Hsin Su,<sup>†</sup> Atallah F. Ahmed,<sup>†,‡</sup> Ping-Jyun Sung,<sup>§</sup> Yang-Chang Wu,<sup>⊥</sup> and Jyh-Horng Sheu<sup>\*,†</sup>

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Eight new meroditerpenoid-related metabolites, including one naphthoquinone derivative, chabrolonaphthoquinone B (**1**), four tetraprenyltoluquinone-related compounds, chabrolbenzoquinones E–H (**2–5**), and three tetraprenyltoluquinol-related metabolites, chabrohydroxybenzoquinones E–G (**6–8**), were isolated from the organic extract of a Taiwanese soft coral *Nephthea chabrolii*. The structures of **1–8** were elucidated on the basis of extensive spectroscopic analysis and by comparison of the data with those of the related metabolites. Cytotoxic activity of metabolites **1–3** and **5–8** against a limited panel of cancer cell lines is also described.

The soft coral *Nephthea chabrolii* Audouin (Alcyonacea, Nephthedae) has afforded several types of metabolites including cembranes and norditerpenes,<sup>1</sup> polyhydroxysteroids,<sup>2</sup> and sesquiterpenes.<sup>3–5</sup> Our previous chemical investigation on *N. chabrolii* had led to the isolation of nine new meroditerpenoids, chabrolonaphthoquinone A, chabrohydroxybenzoquinones A–D, and chabrolbenzoquinones A–D.<sup>6</sup> In this paper, we further report the isolation of eight new meroditerpenes, including one new naphthoquinone derivative, chabrolonaphthoquinone B (**1**), four tetraprenyltoluquinone-related metabolites, chabrolbenzoquinones E–H (**2–5**), and three tetraprenyltoluquinol-related metabolites, chabrohydroxybenzoquinones E–G (**6–8**). The structures of metabolites **1–8** were characterized by extensive spectroscopic analysis and by comparison of the data with those of related metabolites. The cytotoxicity of these meroditerpenoid-related metabolites, except **4**, against human hepatocellular carcinoma (Hep G2), human lung carcinoma (A-549), and breast carcinoma (MDA-MB-231) cell lines was evaluated.

## Results and Discussion

Frozen organisms of *N. chabrolii* were extracted with EtOH. The residue of the EtOH extract was triturated sequentially with *n*-hexane and EtOAc. The EtOAc-soluble fraction was concentrated and fractionated over Si gel gravity column chromatography, and the eluted fractions were further purified by normal-phase HPLC to yield **1–8** (see Experimental Section).

Chabrolonaphthoquinone B (**1**) was isolated as a pale yellow oil. Its molecular formula, C<sub>29</sub>H<sub>38</sub>O<sub>5</sub>, was established by HREIMS (*m/z* 466.2718). The EIMS of **1** showed peaks at *m/z* 466 (M)<sup>+</sup>, 406 (M – HOAc)<sup>+</sup>, and 388 (M – HOAc – H<sub>2</sub>O)<sup>+</sup>, suggesting the presence of an acetoxyl and a hydroxyl in **1**. The <sup>13</sup>C NMR data of **1** (Table 1) in CDCl<sub>3</sub> showed the presence of 29 carbon signals, which were identified by the assistance of a DEPT spectrum as six methyls, six sp<sup>3</sup> methylenes, two oxygenated sp<sup>3</sup> carbons, six sp<sup>2</sup> methines, and nine sp<sup>2</sup> quaternary carbons includ-

Table 1. <sup>1</sup>H and <sup>13</sup>C NMR Data for Compound **1**

C/H	δ <sub>H</sub> <sup>a</sup>	δ <sub>C</sub> <sup>b</sup>
1'		186.0 (C) <sup>d</sup>
2'		148.0 (C)
3'	6.81 d (1.0) <sup>c</sup>	135.8 (CH)
4'		185.0 (C)
4a'		130.3 (C)
5'	7.96 d (8.0)	126.3 (CH)
6'	7.53 dd (8.0, 1.5)	134.0 (CH)
7'		148.9 (C)
8'	7.90 d (1.5)	126.5 (CH)
8a'		132.0 (C)
9'	2.18 d (1.0)	16.5 (CH <sub>3</sub> )
1	2.78 t (7.5)	36.1 (CH <sub>2</sub> )
2	2.35 m	29.3 (CH <sub>2</sub> )
3	5.16 t (7.0)	123.3 (CH)
4		135.9 (C)
5	1.95 m	36.1 (CH <sub>2</sub> )
6	1.69 m	27.6 (CH <sub>2</sub> )
7	4.82 dd (10.5, 2.5)	78.9 (CH)
8		74.2 (C)
9	1.43 m; 1.54 m	37.7 (CH <sub>2</sub> )
10	2.04 m	22.1 (CH <sub>2</sub> )
11	5.11 t (7.0)	124.2 (CH)
12		132.1 (C)
13	1.69 s	25.7 (CH <sub>3</sub> )
14	1.62 s	17.7 (CH <sub>3</sub> )
15	1.16 s	23.5 (CH <sub>3</sub> )
16	1.50 s	16.0 (CH <sub>3</sub> )
OAC	2.10 s	21.1 (CH <sub>3</sub> )
OH	4.75 s	171.1 (C)

<sup>a</sup> Spectra recorded at 500 MHz in CDCl<sub>3</sub>. <sup>b</sup> Spectra recorded at 125 MHz in CDCl<sub>3</sub>. <sup>c</sup> *J* values (in Hz) in parentheses. <sup>d</sup> Attached protons were deduced by DEPT experiments.

ing those of two ketone carbonyls and one ester carbonyl. The signals appearing at δ 186.0, 185.0, 148.9, 148.0, 132.0, 130.3 (each C), 135.8, 134.0, 126.5, 126.3 (each CH), and 16.5 (CH<sub>3</sub>) suggested the presence of one methylated 1,4-naphthoquinone moiety by comparison of the above data with the <sup>13</sup>C NMR data of the known metabolite **9**.<sup>6</sup> Also, the EIMS ion at *m/z* 185 (C<sub>12</sub>H<sub>9</sub>O<sub>2</sub>)<sup>+</sup> together with the UV absorptions at 343, 266, and 257 nm further confirmed the presence of this moiety.<sup>6</sup> From the <sup>1</sup>H NMR spectrum of **1**, the resonances of four aromatic protons (δ 7.96, d, *J* = 8.0 Hz; 7.90, d, *J* = 1.5 Hz; 7.53, dd, *J* = 8.0, 1.5 Hz; 6.81, d, *J* = 1.0 Hz), two olefinic protons (δ 5.16, t, *J* = 7.0 Hz; 5.11, t, *J* = 7.0 Hz), one oxygenated methine proton (δ 4.82, dd, *J* = 10.5, 2.5 Hz), and six methyls (δ 2.18, d, *J* = 1.0 Hz; 2.10, s; 1.69, s; 1.62, s; 1.50, s; 1.16, s) were observed.

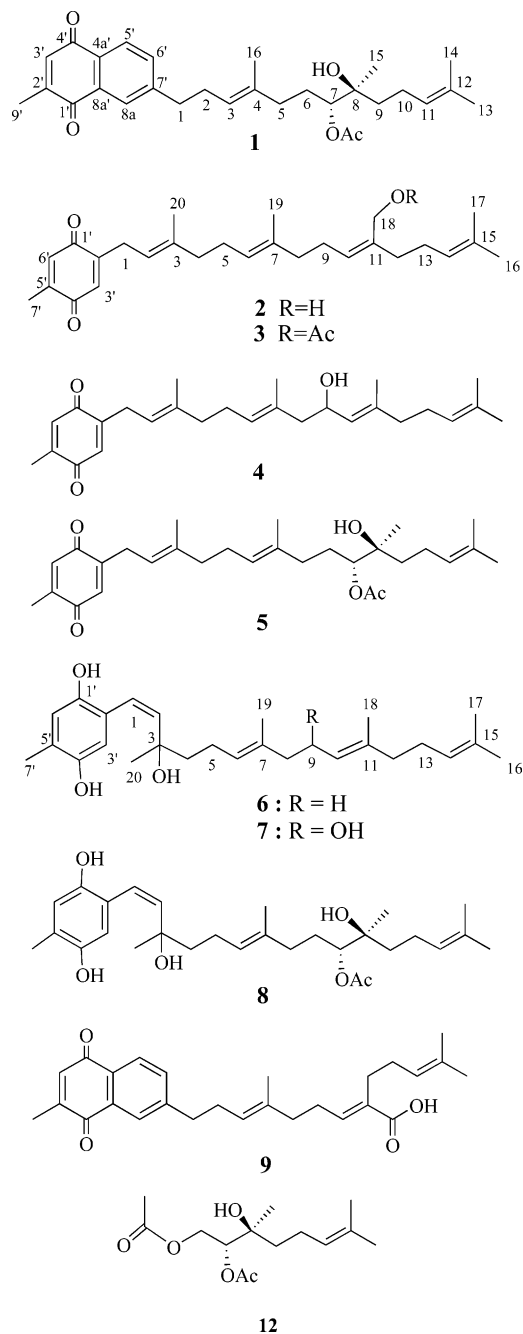
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<sup>†</sup> National Sun Yat-sen University.

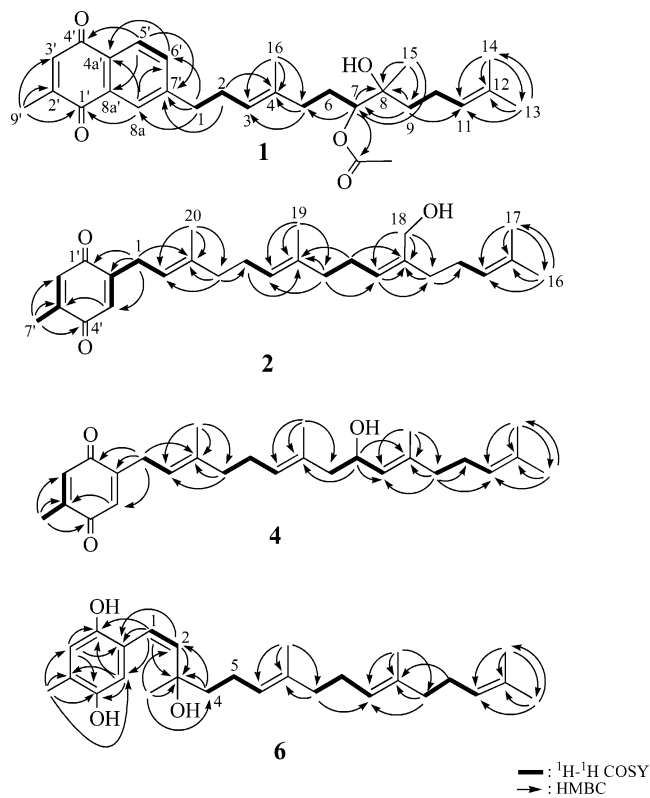
<sup>‡</sup> Mansoura University.

<sup>§</sup> National Museum of Marine Biology and Aquarium.

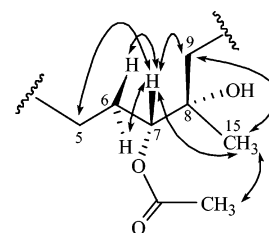
<sup>⊥</sup> Kaohsiung Medical University.



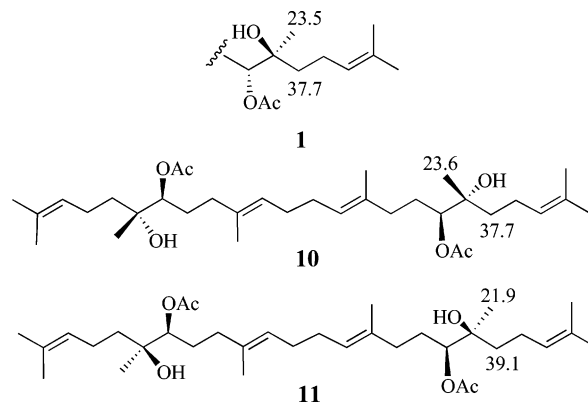
The constitution of the side chain was elucidated initially by the  $^1\text{H}$ - $^1\text{H}$  COSY correlations (Figure 1) from H<sub>2</sub>-1 to H-3, H<sub>2</sub>-5 to H-7, and H<sub>2</sub>-9 to H-11 and by the key HMBC correlations (Figure 1) from H<sub>2</sub>-2 to C-4; H<sub>2</sub>-5 to C-3, C-4; H-7 to C-5, C-8; H<sub>2</sub>-9 to C-7, C-8, C-11; H<sub>3</sub>-13 to C-11, C-12, C-14; and H<sub>3</sub>-14 to C-11, C-12, C-13. Thus, the connectivity from C-1 to C-14 was fully established. The methyl groups attached at C-4 and C-8 were then confirmed by the HMBC correlations from H<sub>3</sub>-15 to C-7, C-8, C-9 and H<sub>3</sub>-16 to C-3, C-4, C-5, respectively. One acetoxy group positioned at C-7 was confirmed by the HMBC correlation between an oxymethine proton resonating at  $\delta$  4.82 (H-7) and the ester carbonyl carbon at  $\delta$  171.1. Furthermore, the position of this prenylated side chain at C-7' was established from the HMBC correlations from H<sub>2</sub>-1 to C-6', C-7', C-8' and H<sub>2</sub>-2 to C-7'. The geometry of the double bond between C-3 and C-4 was shown to be *E*, by comparison of the NMR spectral data with those of **9**.<sup>6</sup> These data, together with other HMBC correlations (Figure 1), unambiguously established the molecular framework of **1**. Moreover, the NOE correla-



**Figure 1.** Key  $^1\text{H}$ - $^1\text{H}$  COSY and HMBC correlations for **1**, **2**, **4**, and **6**.



**Figure 2.** Selective NOE correlations of **1**.



**Figure 3.** Carbon shifts of C-9 and C-15 of **1** relative to those of C-15 and C-14 of squalene derivatives **10** and **11**.

tions from a NOESY experiment revealed the following key interactions: H-7/H<sub>2</sub>-5, H-7/H<sub>2</sub>-6, H-7/H<sub>2</sub>-9, H-7/H<sub>3</sub>-15, H<sub>3</sub>-15/H<sub>2</sub>-9, and H<sub>3</sub>-15/H<sub>3</sub>-OAc. Consideration of molecular models revealed that the partial structure shown in Figure 2 may fit the above NOE correlations. Also, by comparison of the carbon shifts of C-9 and C-15 of **1** with those of the environmentally similar carbons of the two squalene-derived compounds **10** and **11**,<sup>7</sup> it was suggested that **1** should possess a 10,11-*erythro* relative configuration (Figure 3). Furthermore, metabolite **1** ( $[\alpha]^{25}_{\text{D}} -19.3^\circ$ ) has

**Table 2.** <sup>1</sup>H NMR Chemical Shifts for Compounds 2–8

	2 <sup>a</sup>	3 <sup>a</sup>	4 <sup>a</sup>	5 <sup>b</sup>	6 <sup>a</sup>	7 <sup>a</sup>	8 <sup>a</sup>
3'	6.50 s	6.50 s	6.51 s	6.50 s	6.42 s	6.43 s	6.42 s
6'	6.59 q (1.5) <sup>c</sup>	6.59 q (1.5)	6.59 q (1.5)	6.60 q (1.5)	6.56 s	6.57 s	6.55 s
7'	2.03 d (1.5)	2.04 d (1.5)	2.03 d (1.5)	2.04 d (1.5)	2.18 s	2.18 s	2.17 s
1	3.11 d (7.5)	3.11 d (7.5)	3.11 d (7.0)	3.11 d (7.2)	6.26 d (10.0)	6.27 d (10.0)	6.24 d (10.0)
2	5.15 t (7.5)	5.15 t (7.5)	5.15 m <sup>d</sup>	5.13 t (7.2)	5.54 d (10.0)	5.53 d (10.0)	5.52 d (10.0)
4	2.08 m	2.08 m	2.08 m	2.09 m	1.64 m; 1.71 m	1.65 m; 1.72 m	1.63 m; 1.70 m
5	2.12 m	2.12 m	2.14 m	2.12 m	2.11 m	2.14 m	2.10 m
6	5.12 m <sup>d</sup>	5.11 m <sup>d</sup>	5.22 t (7.0)	5.11 m <sup>d</sup>	5.11 m	5.25 t (6.8)	5.10 t (7.0)
8	2.02 m	2.02 m	2.10 m	1.96 m	1.98 m	2.12 m	1.96 m
9	2.16 m	2.17 m	4.43 m	1.71 m	2.06 m	4.43 m	1.68 m
10	5.31 t (7.0)	5.41 t (7.5)	5.16 m <sup>d</sup>	4.85 dd (9.6, 3.0)	5.10 m <sup>d</sup>	5.16 d (9.0)	4.82 dd (10.0, 2.0)
12	2.13 m	2.09 m	2.00 m	1.43 m; 1.54 m	1.95 m	2.00 m	1.43 m; 1.54 m
13	2.12 m	2.08 m	2.09 m	2.05 m	2.08 m	2.08 m	2.03 m
14	5.11 m <sup>d</sup>	5.09 m <sup>d</sup>	5.09 t (7.0)	5.11 m <sup>d</sup>	5.10 m <sup>d</sup>	5.09 t (7.0)	5.10 t (7.0)
16	1.69 s	1.68 s	1.68 s	1.68 s	1.68 s	1.68 s	1.68 s
17	1.61 s	1.60 s	1.60 s	1.62 s	1.60 s	1.60 s	1.62 s
18	4.12 s	4.59 s	1.68 s	1.17 s	1.58 s	1.67 s	1.16 s
19	1.61 s	1.60 s	1.67 s	1.60 s	1.58 s	1.63 s	1.56 s
20	1.62 s	1.62 s	1.63 s	1.62 s	1.36 s	1.36 s	1.35 s
OAc		2.07 s		2.11 s			2.09 s

<sup>a</sup> Spectra recorded at 500 MHz in CDCl<sub>3</sub>. <sup>b</sup> Spectra recorded at 300 MHz in CDCl<sub>3</sub>. <sup>c</sup> *J* values (in Hz) in parentheses. <sup>d</sup> Interchangeable values.

**Table 3.** <sup>13</sup>C NMR Chemical Shifts for Compounds 2–8

	2 <sup>a</sup>	3 <sup>a</sup>	4 <sup>a</sup>	5 <sup>b</sup>	6 <sup>a</sup>	7 <sup>a</sup>	8 <sup>a</sup>
1'	187.9 (C) <sup>c</sup>	187.9 (C)	187.8 (C)	187.9 (C)	146.7 (C)	146.7 (C)	146.6 (C)
2'	148.5 (C)	148.4 (C)	148.3 (C)	148.6 (C)	119.6 (C)	119.5 (C)	119.5 (C)
3'	132.3 (CH)	132.3 (CH)	132.3 (CH)	132.4 (CH)	112.4 (CH)	112.5 (CH)	112.4 (CH)
4'	188.4 (C)	188.4 (C)	188.4 (C)	188.4 (C)	147.3 (C)	147.4 (C)	147.5 (C)
5'	145.6 (C)	145.6 (C)	145.6 (C)	145.7 (C)	124.4 (C)	124.5 (C)	124.6 (C)
6'	133.5 (CH)	133.5 (CH)	133.5 (CH)	133.6 (CH)	118.1 (CH)	118.2 (CH)	118.1 (CH)
7'	15.5 (CH <sub>3</sub> )	15.5 (CH <sub>3</sub> )	15.5 (CH <sub>3</sub> )	15.5 (CH <sub>3</sub> )	15.9 (CH <sub>3</sub> )	16.1 (CH <sub>3</sub> )	15.9 (CH <sub>3</sub> )
1	27.1 (CH <sub>2</sub> )	27.1 (CH <sub>2</sub> )	27.1 (CH <sub>2</sub> )	27.2 (CH <sub>2</sub> )	122.4 (CH)	122.6 (CH)	122.5 (CH)
2	118.0 (CH)	117.9 (CH)	118.3 (CH)	118.0 (CH)	129.8 (CH)	129.6 (CH)	129.7 (CH)
3	139.7 (C)	139.9 (C)	139.6 (C)	139.8 (C)	78.0 (C)	77.9 (C)	77.9 (C)
4	39.5 (CH <sub>2</sub> )	39.6 (CH <sub>2</sub> )	39.4 (CH <sub>2</sub> )	39.6 (CH <sub>2</sub> )	40.9 (CH <sub>2</sub> )	40.8 (CH <sub>2</sub> )	40.8 (CH <sub>2</sub> )
5	26.2 (CH <sub>2</sub> )	26.4 (CH <sub>2</sub> )	26.3 (CH <sub>2</sub> )	26.4 (CH <sub>2</sub> )	22.6 (CH <sub>2</sub> )	22.8 (CH <sub>2</sub> )	22.8 (CH <sub>2</sub> )
6	124.3 (CH)	124.3 (CH)	127.9 (CH)	124.3 (CH)	124.0 (CH)	128.4 (CH)	124.7 (CH)
7	135.0 (C)	134.8 (C)	132.1 (C)	134.6 (C)	135.2 (C)	131.8 (C)	134.3 (C)
8	39.8 (CH <sub>2</sub> )	39.6 (CH <sub>2</sub> )	48.1 (CH <sub>2</sub> )	36.2 (CH <sub>2</sub> )	39.7 (CH <sub>2</sub> )	48.1 (CH <sub>2</sub> )	36.1 (CH <sub>2</sub> )
9	26.2 (CH <sub>2</sub> )	26.3 (CH <sub>2</sub> )	65.8 (CH)	27.7 (CH <sub>2</sub> )	26.6 (CH <sub>2</sub> )	65.9 (CH)	27.5 (CH <sub>2</sub> )
10	128.5 (CH)	130.7 (CH)	127.2 (CH)	79.1 (CH)	124.2 (CH)	127.2 (CH)	79.1 (CH)
11	138.4 (C)	133.5 (C)	138.1 (C)	74.2 (C)	134.9 (C)	138.2 (C)	74.3 (C)
12	35.2 (CH <sub>2</sub> )	35.2 (CH <sub>2</sub> )	39.5 (CH <sub>2</sub> )	37.8 (CH <sub>2</sub> )	39.7 (CH <sub>2</sub> )	39.5 (CH <sub>2</sub> )	37.5 (CH <sub>2</sub> )
13	27.1 (CH <sub>2</sub> )	26.8 (CH <sub>2</sub> )	26.4 (CH <sub>2</sub> )	22.1 (CH <sub>2</sub> )	26.7 (CH <sub>2</sub> )	26.4 (CH <sub>2</sub> )	22.0 (CH <sub>2</sub> )
14	124.2 (CH)	123.9 (CH)	124.0 (CH)	124.5 (CH)	124.4 (CH)	124.0 (CH)	124.1 (CH)
15	131.7 (C)	131.7 (C)	131.6 (C)	132.1 (C)	131.3 (C)	131.6 (C)	132.1 (C)
16	25.7 (CH <sub>3</sub> )	25.7 (CH <sub>3</sub> )	25.7 (CH <sub>3</sub> )	25.8 (CH <sub>3</sub> )	25.7 (CH <sub>3</sub> )	25.7 (CH <sub>3</sub> )	25.7 (CH <sub>3</sub> )
17	17.7 (CH <sub>3</sub> )	17.7 (CH <sub>3</sub> )	17.7 (CH <sub>3</sub> )	17.7 (CH <sub>3</sub> )	17.7 (CH <sub>3</sub> )	17.7 (CH <sub>3</sub> )	17.7 (CH <sub>3</sub> )
18	60.3 (CH <sub>2</sub> )	62.1 (CH <sub>2</sub> )	16.6 (CH <sub>3</sub> )	23.6 (CH <sub>3</sub> )	16.0 (CH <sub>3</sub> )	16.6 (CH <sub>3</sub> )	23.6 (CH <sub>3</sub> )
19	16.1 (CH <sub>3</sub> )	16.0 (CH <sub>3</sub> )	16.2 (CH <sub>3</sub> )	16.2 (CH <sub>3</sub> )	16.0 (CH <sub>3</sub> )	15.9 (CH <sub>3</sub> )	15.9 (CH <sub>3</sub> )
20	16.1 (CH <sub>3</sub> )	16.1 (CH <sub>3</sub> )	16.0 (CH <sub>3</sub> )	16.1 (CH <sub>3</sub> )	26.0 (CH <sub>3</sub> )	26.1 (CH <sub>3</sub> )	26.0 (CH <sub>3</sub> )
OAc		21.0 (CH <sub>3</sub> )		21.1 (CH <sub>3</sub> )			21.1 (CH <sub>3</sub> )
		171.2 (C)		171.1 (C)			171.2 (C)

<sup>a</sup> Spectra recorded at 125 MHz in CDCl<sub>3</sub>. <sup>b</sup> Spectra recorded at 75 MHz in CDCl<sub>3</sub>. <sup>c</sup> Attached protons were deduced by DEPT experiments.

the same sign of specific rotation as that of the synthetic monoterpene **12** ( $[\alpha]_D -11.5^\circ$ ).<sup>8</sup> Thus, the absolute configuration of **1** was assumed to be 7*R*, 8*S*. On the basis of above analysis, the structure of **1** was established.

Chabrolbenzoquinone F (**2**) was isolated as a pale yellow oil that gave an  $[M + Na]^+$  ion peak at 433.2715 *m/z* in the HRESIMS, appropriate for a molecular formula of C<sub>27</sub>H<sub>38</sub>O<sub>3</sub> requiring nine degrees of unsaturation. The presence of a hydroxy group in **2** was revealed from the absorption band at 3468 cm<sup>-1</sup> and the ion peak at *m/z* 392  $[M - H_2O]^+$  in the IR and EIMS spectra, respectively. Moreover, the UV ( $\lambda_{max}$  252 nm) and IR ( $\nu_{max}$  1657 and 1610 cm<sup>-1</sup>) absorption bands were characteristic for benzoquinones.<sup>6,9,10</sup> From the <sup>1</sup>H and <sup>13</sup>C NMR data (Tables 2 and 3), in combination with the HMQC data, 27 carbon signals were assigned to five methyls, eight sp<sup>3</sup> methylenes, six

sp<sup>2</sup> methines, six sp<sup>2</sup> quaternary olefinic carbons, and two carbonyls. The <sup>1</sup>H NMR spectrum of **2** showed signals of two quinone protons ( $\delta$  6.59, q, *J* = 1.5 Hz; 6.50, s), four olefinic protons ( $\delta$  5.31, t, *J* = 7.0 Hz; 5.15, t, *J* = 7.5 Hz; 5.12, m; 5.11, m), one oxygen-bearing methylene ( $\delta$  4.12, 2H, s), and five methyls ( $\delta$  2.03, d, *J* = 1.5 Hz; 1.69, s; 1.62, s each 3H; 1.61, 6H, s). The <sup>1</sup>H–<sup>1</sup>H COSY correlations (Figure 1) showed allylic coupling between H<sub>3</sub>–7' and H–6' and between H–3' and H<sub>2</sub>–1, and HMBC data (Figure 1) showed correlations between H<sub>2</sub>–1 and C–1', C–2', C–3'; H–3' and C–5'; and H<sub>3</sub>–7' and C–4', C–5', C–6', establishing the 5'-methylquinone moiety of **2**. The structure of the tetraprenylated side chain was established by the <sup>1</sup>H–<sup>1</sup>H COSY correlations from H<sub>2</sub>–1 to H–2, H<sub>2</sub>–5 to H–6, H<sub>2</sub>–8 to H–10, and H<sub>2</sub>–13 to H–14 and HMBC correlations from H<sub>2</sub>–1 to C–3; H<sub>2</sub>–4 to C–2, C–3, C–5; H<sub>2</sub>–5 to C–7; H<sub>2</sub>–8 to C–6, C–7,

C-10; H<sub>2</sub>-9 to C-7, C-11; H-10 to C-11, C-12; H<sub>2</sub>-12 to C-11, C-13; and H<sub>3</sub>-16 to C-14, C-15 (Figure 1). The methyl groups attached at C-3 and C-7 were further confirmed by the HMBC correlations between H<sub>3</sub>-20 and C-2, C-3, C-4 and H<sub>3</sub>-19 and C-6, C-7, C-8. Also, the oxygen-bearing methylene attached at C-11 was established by the HMBC correlations between H<sub>2</sub>-18 and C-10, C-11, C-12. The geometries of both C<sub>2</sub>-C<sub>3</sub> and C<sub>6</sub>-C<sub>7</sub> double bonds were shown to be *E* by comparison of the NMR data with those of chabrolbenzoquinones A–D.<sup>6</sup> Furthermore, the NOESY spectrum showed correlation of H<sub>2</sub>-18 with H<sub>2</sub>-9, but not with H-10, revealing the *Z* geometry of the C-10/C-11 double bond. On the basis of the above observations, the structure of **2** was established unambiguously.

A structurally similar metabolite, chabrolbenzoquinone F (**3**), was also isolated as a pale yellow oil. Its molecular formula, C<sub>29</sub>H<sub>40</sub>O<sub>4</sub>, was established by HRESIMS (475.2826 *m/z*, [M + Na]<sup>+</sup>), with an additional degree of unsaturation relative to that of **2**. The <sup>1</sup>H and <sup>13</sup>C NMR data (Tables 2 and 3) revealed that **3** is simply the 18-*O*-acetyl derivative of **2**.

Chabrolbenzoquinone G (**4**), obtained as a pale yellow oil, has the same molecular formula, C<sub>27</sub>H<sub>38</sub>O<sub>3</sub>, as that of **2**, as revealed from the EIMS (*m/z* 410, [M]<sup>+</sup>) and NMR data. The IR spectrum exhibited an absorption at 3476 cm<sup>-1</sup> and EIMS showed an ion at *m/z* 392 [M - H<sub>2</sub>O]<sup>+</sup>, suggesting the presence of a hydroxy group in **4**. The <sup>1</sup>H and <sup>13</sup>C NMR spectra also revealed that **4** is a benzoquinone-type compound. By means of extensive 2D NMR experiments (COSY, HMQC, and HMBC), the structure of **4** was found to be close to that of **2** except that the C-9 methylene and the C-18 hydroxymethylene in **2** were replaced by a hydroxymethine and a methyl, respectively. Confirmation of the position of the hydroxy group came from HMBC correlations (Figure 1) observed from H-9 (δ 4.43, m) to C-10 (δ 127.2, CH), C-11 (δ 138.1, C), and C-7 (δ 132.1, C). In addition, the <sup>13</sup>C NMR signal for the oxygen-bearing methylene C-18 (δ 60.3) in **2** was absent and replaced by the signal of a methyl carbon (δ 16.6) in **4**. The geometries of the double bonds between C-2 and C-3, C-6 and C-7, and C-10 and C-11 were all *E*, as the chemical shifts for C-18, C-19, and C-20 were upfield shifted to 16.0–16.6 ppm, in comparison with that of C-16, which resonated at δ 25.7 ppm.

The new metabolite chabrolbenzoquinone H (**5**) was isolated as a pale yellow oil. Its molecular formula, C<sub>29</sub>H<sub>42</sub>O<sub>5</sub>, was established by HREIMS (470.3017 *m/z*, [M]<sup>+</sup>). The <sup>13</sup>C NMR spectrum of **5** (Tables 2 and 3) showed the presence of 29 carbons, and the chemical shifts (δ<sub>H</sub> and δ<sub>C</sub>) of the partial structures (C-1' to C-7'; C-1 to C-8; C-12 to C-17) of **5** were close to those of compounds **2**–**4**. The chemical shifts of the side chain from C-6 to C-19 in **5** are nearly identical with those of **1**. Moreover, **5** has the same sign and close magnitude in specific rotation ([α]<sub>D</sub><sup>25</sup> -20.5°) relative to that of **1**. On the basis of the above observations, the structure of compound **5** was established.

Chabrolhydroxybenzoquinone E (**6**) was isolated as a pale yellow oil and possesses the molecular formula C<sub>27</sub>H<sub>40</sub>O<sub>3</sub>, as revealed by its HRESIMS and <sup>1</sup>H and <sup>13</sup>C NMR data (Tables 2 and 3). The <sup>13</sup>C NMR spectrum exhibited seven signals for a 1,4-dihydroxy-5-methylbenzene subunit (δ 146.7 C, 119.6 C, 112.4 CH, 147.3 C, 124.4 C, 118.1 CH, and 15.9 CH<sub>3</sub>)<sup>6</sup> and eight olefinic carbons of the side chain. Moreover, five additional methyls, six methylenes, and one quaternary carbon were observed. From the <sup>1</sup>H–<sup>1</sup>H COSY spectrum of **6** (Figure 1), the proton sequences from H-1 to H-2, H<sub>2</sub>-4 to H-6, H<sub>2</sub>-8 to H-10, and

**Table 4.** Cytotoxicities of Compounds **1**–**3** and **5**–**8**

compound	cancer cell line (IC <sub>50</sub> , μM)		
	Hep G2	A549	MDA-MB-231
<b>1</b>	12.4	33.9	4.7
<b>2</b>	>48.8	>48.8	>48.8
<b>3</b>	38.1	38.1	33.2
<b>5</b>	>42.5	38.0	31.4
<b>6</b>	>48.5	>48.5	>48.5
<b>7</b>	44.4	42.3	>46.7
<b>8</b>	18.4	26.8	18.0
doxorubicin	0.17	0.17	0.07

H<sub>2</sub>-12 to H-14 could be established. On the basis of these data and the <sup>1</sup>H/<sup>13</sup>C long-range correlations observed in an HMBC experiment, the connectivities from C-1' to C-7' and from C-1 to C-20 (Figure 1) could be established. The *Z* geometry of the C-1/C-2 double bond was indicated by a 10.0 Hz coupling constant between H-1 and H-2. The *E*-configurations of two double bonds (C-6/C-7 and C-10/C-11) in **6** were assigned on the basis of the <sup>13</sup>C NMR chemical shifts at C-18 (δ 16.0) and C-19 (δ 16.0). Thus, the structure of compound **6** was established.

A structurally similar metabolite **7** was also obtained as a pale yellow oil. The HRESIMS (*m/z* 433.2715, [M - H<sub>2</sub>O + Na]<sup>+</sup>) and NMR data of chabrolhydroxybenzoquinone F (**7**) indicated the molecular formula C<sub>27</sub>H<sub>40</sub>O<sub>4</sub>. Comparison of the <sup>1</sup>H and <sup>13</sup>C NMR data (Tables 2 and 3) of both compounds showed that the structure of **7** should be very close to that of **6** with the exception of signals assigned to C-9, where a methylene (δ<sub>H</sub> 2.06, 2H, m; δ<sub>C</sub> 26.6) in **6** was replaced by a hydroxymethine (δ<sub>H</sub> 4.43, 1H, m; δ<sub>C</sub> 65.9) in **7**. The observed COSY correlation from H-9 to H<sub>2</sub>-8 and H-10 further confirmed the C-9 location of the hydroxy group. Thus, **7** is the 9-hydroxy derivative of **6**.

Chabrolhydroxybenzoquinone G (**8**) is an optically active oil ([α]<sub>D</sub><sup>25</sup> -6.5°). Its molecular formula, C<sub>29</sub>H<sub>44</sub>O<sub>6</sub>, was established by HREIMS and NMR data (Tables 2 and 3). The data of **8** (IR, UV, <sup>1</sup>H and <sup>13</sup>C NMR) are similar to those of **6**; however, an acetoxy group (δ<sub>H</sub> 2.09, 3H, s; δ<sub>C</sub> 21.1 and 171.2) was present in **8**. Furthermore, the <sup>13</sup>C NMR signals for the double bond between C-10 (δ 124.2, CH) and C-11 (δ 134.9, C) in **6** were replaced by the signals of two oxygenated carbons (δ 79.1, CH; 74.3, C) in **8**. Analysis of the <sup>1</sup>H and <sup>13</sup>C NMR data showed that the partial structure of the side chain from C-5 to C-19 in **8** should be identical to those of **1** and **5**. Thus, the structure of compound **8** was established.

Cytotoxicity of metabolites **1**–**3** and **5**–**8** toward a limited panel of cancer cell lines was evaluated. The results (Table 4) showed that compound **1** exhibited significant cytotoxicity against the growth of the MDA-MB-231 (IC<sub>50</sub> 4.7 μM) cancer cell line and moderate to weak cytotoxicity against Hep G2 (IC<sub>50</sub> 12.4 μM) and A549 (IC<sub>50</sub> 33.9 μM) cancer cell lines, respectively. Also, metabolite **8** exhibited moderate to weak cytotoxicity toward these cancer cells. Other metabolites either were inactive or exhibit only weak cytotoxicity against the growth of the above three cancer cell lines.

## Experimental Section

**General Experimental Procedures.** IR spectra were recorded on a Jasco FT-5300 infrared spectrophotometer. Ultraviolet spectra were recorded on a Hitachi U-3210 UV spectrophotometer. NMR spectra were recorded on a Bruker AVANCE DPX300 FT-NMR at 300 MHz for <sup>1</sup>H and 75 MHz for <sup>13</sup>C or on a Varian Unity INOVA 500 FT-NMR at 500 MHz for <sup>1</sup>H and 125 MHz for <sup>13</sup>C, respectively, in CDCl<sub>3</sub>. Low-resolution MS data were obtained by EI on a VG QUATTRO

GC/MS spectrometer or by ESI on a Bruker APEX II mass spectrometer. HRMS were recorded by ESI or EIMS on a Bruker APEX II mass spectrometer. Silica gel (Merck, 230–400 mesh) was used for column chromatography. Precoated silica gel plates (Merck, Kieselgel 60 F-254, 0.2 mm) were used for analytical TLC. High-performance liquid chromatography (HPLC) was performed on a Hitachi L-7100 apparatus equipped with a Bischoff refractive index detector or a Hitachi L-7400 UV detector and with a Merck Hibar Si-60 column (250 × 21 mm, 7 μm).

**Animal Material.** The soft coral *N. chabrolii* was collected by hand using scuba off the coast of Pingtung County, southern Taiwan, in July 2001, at depths of 15 to 20 m, and stored in a freezer until extraction. A voucher sample was deposited at the Department of Marine and Biotechnology and Resources, Sun Yat-Sen University.

**Extraction and Separation.** The sliced bodies of *N. chabrolii* (1.8 kg, wet wt) were exhaustively homogenized with EtOH and filtered. The ground organism was repeatedly extracted with EtOH. The combined EtOH extract was concentrated under vacuum to afford a dark brown viscous residue (20.8 g). The residue was triturated with *n*-hexane to afford an *n*-hexane-soluble fraction and then with EtOAc. The combined EtOAc-soluble fraction was evaporated under vacuum to yield an oily residue (15.8 g), which was subjected to column chromatography on silica gel, using *n*-hexane, *n*-hexane–EtOAc mixtures of increasing polarity, and finally pure EtOAc, to yield 28 fractions. Fraction 7, eluted with *n*-hexane–EtOAc (15:1), was further purified on silica gel using *n*-hexane–acetone (gradient, 30:1 to 20:1) to yield **3** (2.1 mg). Fraction 10, eluted with *n*-hexane–EtOAc (9:1), was further separated by normal-phase HPLC using *n*-hexane–acetone (12:1) to afford **4** (3.2 mg), **2** (3.2 mg), **5** (3.0 mg), and **6** (6.0 mg). Fraction 13, eluted with *n*-hexane–EtOAc (5:1), was purified by normal-phase HPLC using *n*-hexane–acetone (10:1) to afford **1** (5.0 mg) and **7** (3.0 mg). Fraction 15, eluted with *n*-hexane–EtOAc (4:1), was further purified by normal-phase HPLC using *n*-hexane–acetone (8:1) to afford **8** (10.5 mg).

**Chabrolonaphthoquinone B (1):** pale yellow oil;  $[\alpha]_D^{25}$  –19.3° (*c* 0.88, CHCl<sub>3</sub>); UV (MeOH)  $\lambda_{\max}$  348 (2.64), 265 (3.49), 257 (3.64) nm; IR (neat)  $\nu_{\max}$  3294, 2924, 1732, 1662, 1601 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz) and <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz), see Table 1; EIMS (30 eV) *m/z* 466 (0.6, [M]<sup>+</sup>), 406 (0.6, [M – HOAc]<sup>+</sup>), 388 (0.3, [M – HOAc – H<sub>2</sub>O]<sup>+</sup>), 185 (2); HREIMS *m/z* 466.2718 (calcd for C<sub>29</sub>H<sub>38</sub>O<sub>5</sub>, 466.2720).

**Chabrolbenzoquinone E (2):** pale yellow oil; UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ) 252 (3.89) nm; IR (neat)  $\nu_{\max}$  3468, 2924, 1657, 1610 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz) and <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz), see Tables 2 and 3; EIMS (30 eV) *m/z* 410 (2, [M]<sup>+</sup>), 392 (0.3, [M – H<sub>2</sub>O]<sup>+</sup>), 175 (21), 137 (21), 69 (100); HRESIMS *m/z* 433.2715 (calcd for C<sub>27</sub>H<sub>38</sub>O<sub>3</sub>Na, 433.2720).

**Chabrolbenzoquinone F (3):** pale yellow oil; UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ) 251 (3.95) nm; IR (neat)  $\nu_{\max}$  2926, 1736, 1656, 1635 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz) and <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz), see Tables 2 and 3; ESIMS *m/z* 475 (100, [M + Na]<sup>+</sup>); HRESIMS *m/z* 475.2826 (calcd for C<sub>29</sub>H<sub>40</sub>O<sub>4</sub>Na, 475.2826).

**Chabrolbenzoquinone G (4):** pale yellow oil;  $[\alpha]_D^{25}$  +6.4° (*c* 0.5, CHCl<sub>3</sub>); UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ) 251 (3.99) nm; IR (neat)  $\nu_{\max}$  3476, 2924, 1657, 1614 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz) and <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz), see Tables 2 and 3; EIMS (30 eV) *m/z* 410 (0.2, [M]<sup>+</sup>), 392 (0.5, [M – H<sub>2</sub>O]<sup>+</sup>), 175 (86), 137 (44), 69 (100); HREIMS *m/z* 392.2720 (calcd for C<sub>27</sub>H<sub>38</sub>O<sub>3</sub>, M<sup>+</sup> – H<sub>2</sub>O, 392.2717).

**Chabrolbenzoquinone H (5):** pale yellow oil;  $[\alpha]_D^{25}$  –20.5° (*c* 0.5, CHCl<sub>3</sub>); UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ) 252 (3.95) nm; IR (neat)  $\nu_{\max}$  3393, 2930, 1728, 1641 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz) and <sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz), see Tables 2 and 3; EIMS (70 eV) *m/z* 470 (0.8, [M]<sup>+</sup>), 410 (0.2, [M – HOAc]<sup>+</sup>), 392 (0.1, [M – H<sub>2</sub>O – HOAc]<sup>+</sup>), 175 (71), 69 (100); HREIMS 470.3017 *m/z* (calcd for C<sub>29</sub>H<sub>42</sub>O<sub>5</sub>, 470.3021).

**Chabrolhydroxybenzoquinone E (6):** pale yellow oil; UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ) 331 (3.79), 266 (3.77) nm; IR (neat)  $\nu_{\max}$  3398, 2926, 1684, 1637 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz) and <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz), see Tables 2 and 3; ESIMS *m/z* 417 (100, [M – H<sub>2</sub>O + Na]<sup>+</sup>); HRESIMS 417.2772 *m/z* (calcd for C<sub>27</sub>H<sub>38</sub>O<sub>2</sub>Na, M<sup>+</sup> – H<sub>2</sub>O + Na, 417.2771).

**Chabrolhydroxybenzoquinone F (7):** pale yellow oil;  $[\alpha]_D^{25}$  +1.6° (*c* 1.0, CHCl<sub>3</sub>); UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ) 330 (3.63), 267 (3.62) nm; IR (neat)  $\nu_{\max}$  3472, 2924, 1645 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz) and <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz), see Tables 2 and 3; EIMS (30 eV) *m/z* 410 (0.2, [M – H<sub>2</sub>O]<sup>+</sup>), 392 (0.5, [M – 2H<sub>2</sub>O]<sup>+</sup>), 175 (100), 137 (5), 69 (65); HRESIMS *m/z* 433.2715 (calcd for C<sub>27</sub>H<sub>38</sub>O<sub>3</sub>Na, M<sup>+</sup> – H<sub>2</sub>O + Na, 433.2720).

**Chabrolhydroxybenzoquinone G (8):** pale yellow oil;  $[\alpha]_D^{25}$  –6.5° (*c* 1.08, CHCl<sub>3</sub>); UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ) 331 (3.64), 267 (3.65) nm; IR (neat)  $\nu_{\max}$  3422, 2926, 1716, 1658 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz) and <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz), see Tables 2 and 3; EIMS (30 eV) *m/z* 470 (2, [M – H<sub>2</sub>O]<sup>+</sup>), 410 (0.1, [M – H<sub>2</sub>O – HOAc]<sup>+</sup>), 392 (0.1), 175 (100), 137 (5), 69 (10); HREIMS *m/z* 470.3032 (calcd for C<sub>29</sub>H<sub>42</sub>O<sub>5</sub>, M<sup>+</sup> – H<sub>2</sub>O, 470.3034).

**Cytotoxicity Testing.** Cytotoxicity assays of the test compounds **1–3** and **5–8** were performed using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric method.<sup>11,12</sup>

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