Manaarenolides A–I, Diterpenoids from the Soft Coral Sinularia m anaarensis

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Meroditerpenoids from a Formosan Soft Coral *Nephthea chabrolii*

Jui-Hsin Su,[†] Atallah F. Ahmed,[†,‡] Ping-Jyun Sung,[§] Yang-Chang Wu,[⊥] and Jyh-Horng Sheu*[,†]

Department of Marine Biotechnology and Resources, National Sun Yat-sen University, Kaohsiung 804, Taiwan, Republic of China, Department of Pharmacognosy, Faculty of Pharmacy, Mansoura University, Mansoura 35516, Egypt, National Museum of Marine Biology and Aquarium, Checheng, Pingtung 944, Taiwan, Republic of China, and Institute of Natural Products, Kaohsiung Medical University, Kaohsiung 807, Taiwan, Republic of China

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Eight new meroditerpenoid-related metabolites, including one naphthoquinone derivative, chabrolonaphthoquinone B (**1**), four tetraprenyltoluquinone-related compounds, chabrolobenzoquinones E−H (**2**−**5**), and three tetraprenyltoluquinol-related metabolites, chabrolohydroxybenzoquinones 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,[1] polyhydroxysteroids,[2] and sesquiterpenes.[3−5] Our previous chemical investigation on *N. chabrolii* had led to the isolation of nine new meroditerpenoids, chabrolonaphthoquinone A, chabrolohydroxybenzoquinones A−D, and chabrolobenzoquinones A−D.[6] In this paper, we further report the isolation of eight new meroditerpenes, including one new naphthoquinone derivative, chabrolonaphthoquinone B (**1**), four tetraprenyltoluquinone-related metabolites, chabrolobenzoquinones E−H (**2**−**5**), and three tetraprenyltoluquinol-related metabolites, chabrolohydroxybenzoquinones 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, $C_{29}H_{38}O_5$, was established by HREIMS (*m/z* 466.2718). The EIMS of **1** showed peaks at *m/z* 466 $(M)^+$, 406 $(M - HOAc)^+$, and 388 $(M - HOAc - H_2O)^+$, suggesting the presence of an acetoxyl and a hydroxyl in **1**. The ^{13}C NMR data of **1** (Table 1) in $CDCl_3$ showed the presence of 29 carbon signals, which were identified by the assistance of a DEPT spectrum as six methyls, six sp^3 methylenes, two oxygenated sp^3 carbons, six sp^2 methines, and nine sp^2 quaternary carbons including 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_3) suggested the presence of one methylated 1,4-naphthoquinone moiety by comparison of the above data with the ^{13}C NMR data of the known metabolite **9**.[6] Also, the EIMS ion at *m/z* 185 $(C_{12}H_9O_2)^+$ together with the UV absorptions at 343, 266, and 257 nm further confirmed the presence of this moiety.[6] From the ^1H 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.

Table 1. ^1H and ^{13}C NMR Data for Compound **1**

C/H	δ_H[a]	δ_C[b]
1′		186.0 (C)[d]
2′		148.0 (C)
3′	6.81 d (1.0)[c]	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_3)
1	2.78 t (7.5)	36.1 (CH_2)
2	2.35 m	29.3 (CH_2)
3	5.16 t (7.0)	123.3 (CH)
4		135.9 (C)
5	1.95 m	36.1 (CH_2)
6	1.69 m	27.6 (CH_2)
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_2)
10	2.04 m	22.1 (CH_2)
11	5.11 t (7.0)	124.2 (CH)
12		132.1 (C)
13	1.69 s	25.7 (CH_3)
14	1.62 s	17.7 (CH_3)
15	1.16 s	23.5 (CH_3)
16	1.50 s	16.0 (CH_3)
OAC	2.10 s	21.1 (CH_3)
		171.1 (C)
OH	4.75 s	

[a] Spectra recorded at 500 MHz in $CDCl_3$. [b] Spectra recorded at 125 MHz in $CDCl_3$. [c] J values (in Hz) in parentheses. [d] Attached protons were deduced by DEPT experiments.

* To whom correspondence should be addressed. Tel: +886-7-5252000, ext. 5030. Fax: +886-7-5255020. E-mail: sheu@mail.nsysu.edu.tw.
[†] National Sun Yat-sen University.
[‡] Mansoura University.
[§] National Museum of Marine Biology and Aquarium.
[⊥] Kaohsiung Medical University.

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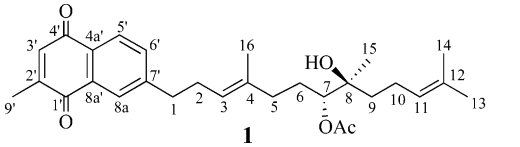

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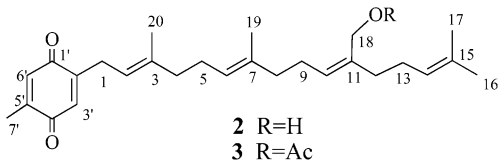

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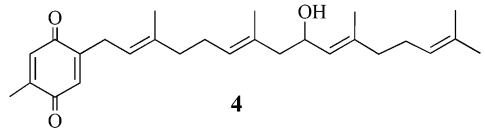
3 R=Ac



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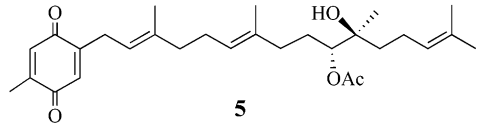

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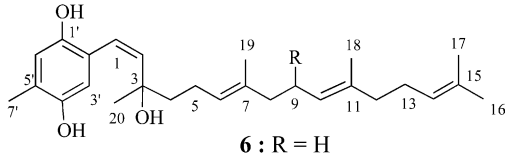
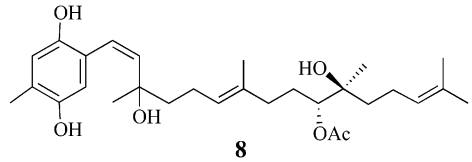


6 : R = H
7 : R = OH



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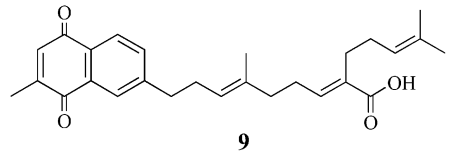

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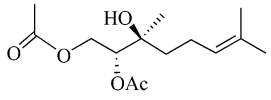

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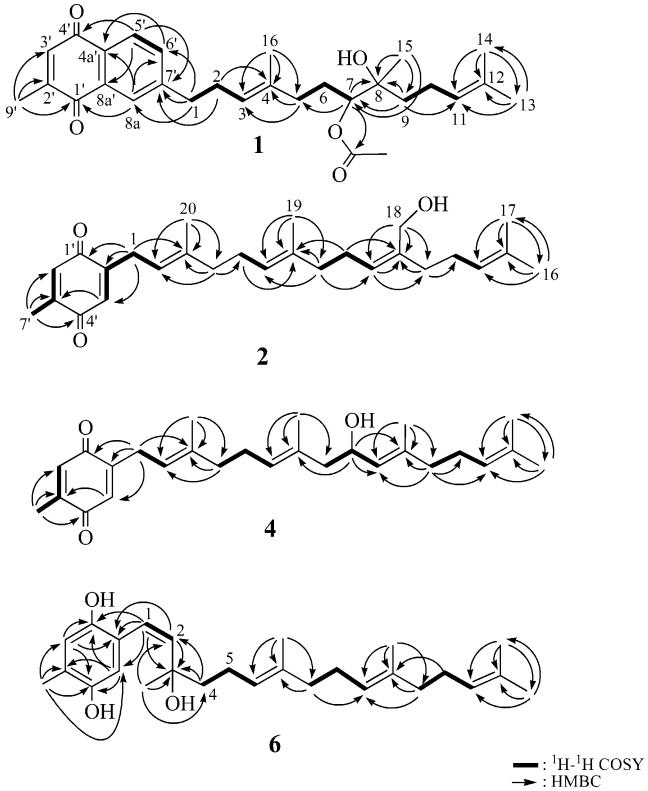
The constitution of the side chain was elucidated initially by the $^1H-^1H$ COSY correlations (Figure 1) from H_2-1 to H-3, H_2-5 to H-7, and H_2-9 to H-11 and by the key HMBC correlations (Figure 1) from H_2-2 to C-4; H_2-5 to C-3, C-4; H-7 to C-5, C-8; H_2-9 to C-7, C-8, C-11; H_3-13 to C-11, C-12, C-14; and H_3-14 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_3-15 to C-7, C-8, C-9 and H_3-16 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 δ 4.82 (H-7) and the ester carbonyl carbon at δ 171.1. Furthermore, the position of this prenylated side chain at C-7′ was established from the HMBC correlations from H_2-1 to C-6′, C-7′, C-8′ and H_2-2 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**.[6] These data, together with other HMBC correlations (Figure 1), unambiguously established the molecular framework of **1**. Moreover, the NOE correlations from a NOESY experiment revealed the following key interactions: H-7/H_2-5, H-7/H_2-6, H-7/H_2-9, H-7/H_3-15, H_3-15/H_2-9, and H_3-15/H_3-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**,[7] it was suggested that **1** should possess a 10,11-*erythreo* relative configuration (Figure 3). Furthermore, metabolite **1** ($[\alpha]^{25}_D$ −19.3°) has



: $^1H-^1H$ COSY
: HMBC

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

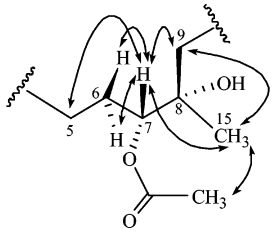


Figure 2. Selective NOE correlations of **1**.

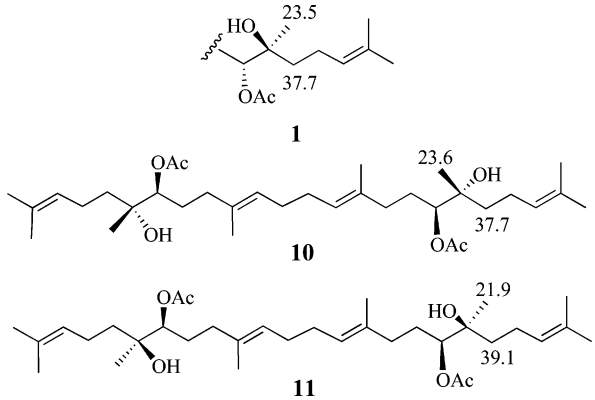


Figure 3. Carbons shifts of C-9 and C-15 of **1** relative to those of C-15 and C-14 of squalene derivatives **10** and **11**.

Table 2. ^{1}H NMR Chemical Shifts for Compounds **2**−**8**

	2[a]	**3**[a]	**4**[a]	**5**[b]	**6**[a]	**7**[a]	**8**[a]
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)[c]	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[d]	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[d]	5.11 m[d]	5.22 t (7.0)	5.11 m[d]	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[d]	4.85 dd (9.6, 3.0)	5.10 m[d]	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[d]	5.09 m[d]	5.09 t (7.0)	5.11 m[d]	5.10 m[d]	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

[a] Spectra recorded at 500 MHz in $CDCl_3$. [b] Spectra recorded at 300 MHz in $CDCl_3$. [c] *J* values (in Hz) in parentheses. [d] Interchangeable values.

Table 3. ^{13}C NMR Chemical Shifts for Compounds **2**−**8**

	2[a]	**3**[a]	**4**[a]	**5**[b]	**6**[a]	**7**[a]	**8**[a]
1′	187.9 (C)[c]	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_3)	15.5 (CH_3)	15.5 (CH_3)	15.5 (CH_3)	15.9 (CH_3)	16.1 (CH_3)	15.9 (CH_3)
1	27.1 (CH_2)	27.1 (CH_2)	27.1 (CH_2)	27.2 (CH_2)	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_2)	39.6 (CH_2)	39.4 (CH_2)	39.6 (CH_2)	40.9 (CH_2)	40.8 (CH_2)	40.8 (CH_2)
5	26.2 (CH_2)	26.4 (CH_2)	26.3 (CH_2)	26.4 (CH_2)	22.6 (CH_2)	22.8 (CH_2)	22.8 (CH_2)
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_2)	39.6 (CH_2)	48.1 (CH_2)	36.2 (CH_2)	39.7 (CH_2)	48.1 (CH_2)	36.1 (CH_2)
9	26.2 (CH_2)	26.3 (CH_2)	65.8 (CH)	27.7 (CH_2)	26.6 (CH_2)	65.9 (CH)	27.5 (CH_2)
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_2)	35.2 (CH_2)	39.5 (CH_2)	37.8 (CH_2)	39.7 (CH_2)	39.5 (CH_2)	37.5 (CH_2)
13	27.1 (CH_2)	26.8 (CH_2)	26.4 (CH_2)	22.1 (CH_2)	26.7 (CH_2)	26.4 (CH_2)	22.0 (CH_2)
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_3)	25.7 (CH_3)	25.7 (CH_3)	25.8 (CH_3)	25.7 (CH_3)	25.7 (CH_3)	25.7 (CH_3)
17	17.7 (CH_3)	17.7 (CH_3)	17.7 (CH_3)	17.7 (CH_3)	17.7 (CH_3)	17.7 (CH_3)	17.7 (CH_3)
18	60.3 (CH_2)	62.1 (CH_2)	16.6 (CH_3)	23.6 (CH_3)	16.0 (CH_3)	16.6 (CH_3)	23.6 (CH_3)
19	16.1 (CH_3)	16.0 (CH_3)	16.2 (CH_3)	16.2 (CH_3)	16.0 (CH_3)	15.9 (CH_3)	15.9 (CH_3)
20	16.1 (CH_3)	16.1 (CH_3)	16.0 (CH_3)	16.1 (CH_3)	26.0 (CH_3)	26.1 (CH_3)	26.0 (CH_3)
OAc		21.0 (CH_3)		21.1 (CH_3)			21.1 (CH_3)
		171.2 (C)		171.1 (C)			171.2 (C)

[a] Spectra recorded at 125 MHz in $CDCl_3$, [b] Spectra recorded at 75 MHz in $CDCl_3$. [c] Attached protons were deduced by DEPT experiments.

the same sign of specific rotation as that of the synthetic monoterpene **12** ($[\alpha]_D$ −11.5°).[8] 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.

Chabrolobenzoquinone 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 $C_{27}H_{38}O_3$ requiring nine degrees of unsaturation. The presence of a hydroxy group in **2** was revealed from the absorption band at 3468 cm^{-1} and the ion peak at *m/z* 392 $[M - H_2O]^+$ in the IR and EIMS spectra, respectively. Moreover, the UV (λ_{max} 252 nm) and IR (ν_{max} 1657 and 1610 cm^{-1}) absorption bands were characteristic for benzoquinones.[6,9,10] From the ^{1}H and ^{13}C NMR data (Tables 2 and 3), in combination with the HMQC data, 27 carbon signals were assigned to five methyls, eight sp^3 methylenes, six sp^2 methines, six sp^2 quaternary olefinic carbons, and two carbonyls. The ^{1}H NMR spectrum of **2** showed signals of two quinone protons (δ 6.59, q, *J* = 1.5 Hz; 6.50, s), four olefinic protons (δ 5.31, t, *J* = 7.0 Hz; 5.15, t, *J* = 7.5 Hz; 5.12, m; 5.11, m), one oxygen-bearing methylene (δ 4.12, 2H, s), and five methyls (δ 2.03, d, *J* = 1.5 Hz; 1.69, s; 1.62, s each 3H; 1.61, 6H, s). The ^{1}H−^{1}H COSY correlations (Figure 1) showed allylic coupling between H_3-7′ and H-6′ and between H-3′ and H_2-1, and HMBC data (Figure 1) showed correlations between H_2-1 and C-1′, C-2′, C-3′; H-3′ and C-5′; and H_3-7′ 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 ^{1}H−^{1}H COSY correlations from H_2-1 to H-2, H_2-5 to H-6, H_2-8 to H-10, and H_2-13 to H-14 and HMBC correlations from H_2-1 to C-3; H_2-4 to C-2, C-3, C-5; H_2-5 to C-7; H_2-8 to C-6, C-7,

C-10; H_2-9 to C-7, C-11; H-10 to C-11, C-12; H_2-12 to C-11, C-13; and H_3-16 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_3-20 and C-2, C-3, C-4 and H_3-19 and C-6, C-7, C-8. Also, the oxygen-bearing methylene attached at C-11 was established by the HMBC correlations between H_2-18 and C-10, C-11, C-12. The geometries of both C_2-C_3 and C_6-C_7 double bonds were shown to be *E* by comparison of the NMR data with those of chabrolobenzoquinones A–D.[6] Furthermore, the NOESY spectrum showed correlation of H_2-18 with H_2-9, 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, chabrolobenzoquinone F (**3**), was also isolated as a pale yellow oil. Its molecular formula, $C_{29}H_{40}O_4$, was established by HRESIMS (475.2826 *m/z*, $[M + Na]^+$), with an additional degree of unsaturation relative to that of **2**. The ^1H and ^{13}C NMR data (Tables 2 and 3) revealed that **3** is simply the 18-*O*-acetyl derivative of **2**.

Chabrolobenzoquinone G (**4**), obtained as a pale yellow oil, has the same molecular formula, $C_{27}H_{38}O_3$, as that of **2**, as revealed from the EIMS (*m/z* 410, $[M]^+$) and NMR data. The IR spectrum exhibited an absorption at 3476 cm^{-1} and EIMS showed an ion at *m/z* 392 $[M - H_2O]^+$, suggesting the presence of a hydroxy group in **4**. The ^1H and ^{13}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 ^{13}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 chabrolobenzoquinone H (**5**) was isolated as a pale yellow oil. Its molecular formula, $C_{29}H_{42}O_5$, was established by HREIMS (470.3017 *m/z*, $[M]^+$). The ^{13}C NMR spectrum of **5** (Tables 2 and 3) showed the presence of 29 carbons, and the chemical shifts (δ_H and δ_C) 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 ($[\alpha]^{25}_D$ −20.5°) relative to that of **1**. On the basis of the above observations, the structure of compound **5** was established.

Chabrolohydroxybenzoquinone E (**6**) was isolated as a pale yellow oil and possesses the molecular formula $C_{27}H_{40}O_3$, as revealed by its HRESIMS and ^1H and ^{13}C NMR data (Tables 2 and 3). The ^{13}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_3)[6] and eight olefinic carbons of the side chain. Moreover, five additional methyls, six methylenes, and one quaternary carbon were observed. From the $^1H-^1H$ COSY spectrum of **6** (Figure 1), the proton sequences from H-1 to H-2, H_2-4 to H-6, H_2-8 to H-10, and H_2-12 to H-14 could be established. On the basis of these data and the $^1H/^{13}C$ 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 ^{13}C NMR chemical shifts at C-18 (δ 16.0) and C-19 (δ 16.0). Thus, the structure of compound **6** was established.

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

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

A structurally similar metabolite **7** was also obtained as a pale yellow oil. The HRESIMS (*m/z* 433.2715, $[M - H_2O + Na]^+$) and NMR data of chabrolohydroxybenzoquinone F (**7**) indicated the molecular formula $C_{27}H_{40}O_4$. Comparison of the ^1H and ^{13}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_H 2.06, 2H, m; δ_C 26.6) in **6** was replaced by a hydroxymethine (δ_H 4.43, 1H, m; δ_C 65.9) in **7**. The observed COSY correlation from H-9 to H_2-8 and H-10 further confirmed the C-9 location of the hydroxy group. Thus, **7** is the 9-hydroxy derivative of **6**.

Chabrolohydroxybenzoquinone G (**8**) is an optically active oil ($[\alpha]^{25}_D$ −6.5°). Its molecular formula, $C_{29}H_{44}O_6$, was established by HREIMS and NMR data (Tables 2 and 3). The data of **8** (IR, UV, ^1H and ^{13}C NMR) are similar to those of **6**; however, an acetoxy group (δ_H 2.09, 3H, s; δ_C 21.1 and 171.2) was present in **8**. Furthermore, the ^{13}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 ^1H and ^{13}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_{50} 4.7 μM) cancer cell line and moderate to weak cytotoxicity against Hep G2 (IC_{50} 12.4 μM) and A549 (IC_{50} 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 ^1H and 75 MHz for ^{13}C or on a Varian Unity INOVA 500 FT-NMR at 500 MHz for ^1H and 125 MHz for ^{13}C, respectively, in $CDCl_3$. 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; $[\alpha]^{25}_D$ −19.3° (*c* 0.88, $CHCl_3$); UV (MeOH) λ_{max} 348 (2.64), 265 (3.49), 257(3.64) nm; IR (neat) ν_{max} 3294, 2924, 1732, 1662, 1601 cm^{-1}; ^1H ($CDCl_3$, 500 MHz) and ^{13}C ($CDCl_3$, 125 MH_Z) NMR, see Table 1; EIMS (30 eV) *m/z* 466 (0.6, $[M]^+$), 406 (0.6, $[M - HOAc]^+$), 388 (0.3, $[M - HOAc - H_2O]^+$), 185 (2); HREIMS *m/z* 466.2718 (calcd for $C_{29}H_{38}O_5$, 466.2720).

Chabrolobenzoquinone E (2): pale yellow oil; UV (MeOH) λ_{max} (log ϵ) 252 (3.89) nm; IR (neat) ν_{max} 3468, 2924, 1657, 1610 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz), see Tables 2 and 3; EIMS (30 eV) *m/z* 410 (2, $[M]^+$), 392 (0.3, $[M - H_2O]^+$), 175 (21), 137 (21), 69 (100); HRESIMS *m/z* 433.2715 (calcd for $C_{27}H_{38}O_3Na$, 433.2720).

Chabrolobenzoquinone F (3): pale yellow oil; UV (MeOH) λ_{max} (log ϵ) 251 (3.95) nm; IR (neat) ν_{max} 2926, 1736, 1656, 1635 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz), see Tables 2 and 3; ESIMS *m/z* 475 (100, $[M + Na]^+$); HRESIMS *m/z* 475.2826 (calcd for $C_{29}H_{40}O_4Na$, 475.2826).

Chabrolobenzoquinone G (4): pale yellow oil; $[\alpha]^{25}_D$ +6.4° (*c* 0.5, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 251 (3.99) nm; IR (neat) ν_{max} 3476, 2924, 1657, 1614 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz), see Tables 2 and 3; EIMS (30 eV) *m/z* 410 (0.2, $[M]^+$), 392 (0.5, $[M - H_2O]^+$), 175 (86), 137 (44), 69 (100); HREIMS *m/z* 392.2720 (calcd for $C_{27}H_{38}O_3$, $M^+ - H_2O$, 392.2717).

Chabrolobenzoquinone H (5): pale yellow oil; $[\alpha]^{25}_D$ −20.5° (*c* 0.5, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 252 (3.95) nm; IR (neat) ν_{max} 3393, 2930, 1728, 1641 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz), see Tables 2 and 3; EIMS (70 eV) *m/z* 470 (0.8, $[M]^+$), 410 (0.2, $[M - HOAc]^+$), 392 (0.1, $[M - H_2O - HOAc]^+$), 175 (71), 69 (100); HREIMS 470.3017 *m/z* (calcd for $C_{29}H_{42}O_5$, 470.3021).

Chabrolohydroxybenzoquinone E (6): pale yellow oil; UV (MeOH) λ_{max} (log ϵ) 331 (3.79), 266 (3.77) nm; IR (neat) ν_{max} 3398, 2926, 1684, 1637 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz), see Tables 2 and 3; ESIMS *m/z* 417 (100, $[M - H_2O + Na]^+$); HRESIMS 417.2772 *m/z* (calcd for $C_{27}H_{38}O_2Na$, $M^+ - H_2O + Na$, 417.2771).

Chabrolohydroxybenzoquinone F (7): pale yellow oil; $[\alpha]^{25}_D$ +1.6° (*c* 1.0, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 330 (3.63), 267 (3.62) nm; IR (neat) ν_{max} 3472, 2924, 1645 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz), see Tables 2 and 3; EIMS (30 eV) *m/z* 410 (0.2, $[M - H_2O]^+$), 392 (0.5 $[M - 2H_2O]^+$), 175 (100), 137 (5), 69 (65); HRESIMS *m/z* 433.2715 (calcd for $C_{27}H_{38}O_3Na$, $M^+ - H_2O + Na$, 433.2720).

Chabrolohydroxybenzoquinone G (8): pale yellow oil; $[\alpha]^{25}_D$ −6.5° (*c* 1.08, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 331 (3.64), 267 (3.65) nm; IR (neat) ν_{max} 3422, 2926, 1716, 1658 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz), see Tables 2 and 3; EIMS (30 eV) *m/z* 470 (2, $[M - H_2O]^+$), 410 (0.1, $[M - H_2O - HOAc]^+$), 392 (0.1), 175 (100), 137 (5), 69 (10); HREIMS *m/z* 470.3032 (calcd for $C_{29}H_{42}O_5$, $M^+ - H_2O$, 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.[11,12]

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