Terpenoid-Related Metabolites from a Formosan Soft Coral *Nephthea chabrolii*

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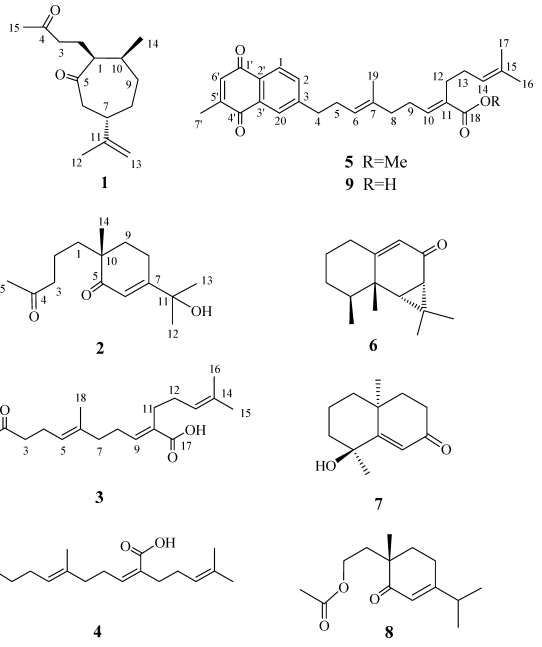
Two new sesquiterpenoidal natural products chabrolidiones A and B (1 and 2), two C_{18} terpenoid-related carboxylic acids, ketochabrolic acid (3) and isoketochabrolic acid (4), and one naphthoquinone derivative chabrolonaphthoquinone C (5), along with two known compounds (+)-aristolone (6) and teuhetenone A (7) were isolated from a Formosan soft coral *Nephthea chabrolii*. The structures of the new metabolites were determined on the basis of extensive spectroscopic analysis and by comparison of NMR data with those of related metabolites. Metabolite 1 has been synthesized previously, but was isolated for the first time from natural sources. Cytotoxic activity of metabolites 1—3 and 5—7 against a limited panel of cancer cell lines is also described.

Key words sesquiterpenoidal natural product; soft coral; *Nephthea chabrolii*

We recently reported a series of new meroditerpenoids including naphthoquinone derivatives, tetraprenyltoluquinone-related metabolites, and tetraprenyltoluquinol-related metabolites from the soft coral *Nephthea chabrolii* Audouin (Alcyonacea, Nephthedae).[1,2] In continuation of our search for cytotoxic metabolites from a soft coral *N. chabrolii*, collected from Taiwanese waters, we have further isolated six new metabolites including two new sesquiterpenoids, chabrolidione A and B (**1** and **2**), two C_{18} terpenoid-related carboxylic acids, ketochabrolic acid (**3**) and isoketochabrolic acid (**4**), and one naphthoquinone derivative chabrolonaphthoquinone C (**5**) along with two known compounds (+)-aristolone (**6**)[3,4] and teuhetenone A (**7**).[5] The structures of **1**—**5** were elucidated on the basis of extensive spectroscopic analyses and by comparison of the spectral data with those of the related metabolites. The relative configuration of **1** was established by careful analysis on the NOE correlations of their NOESY spectra. Cytotoxicity of these compounds toward several cancer cell lines was also evaluated.



Soft corals specimen was collected off the coast of Pingtung county, southern Taiwan, and extracted exhaustively with in EtOH. After evaporation of the solvent, the residue of EtOH extract was triturated sequentially with *n*-hexane, and then with EtOAc. The EtOAc and *n*-hexane extracts were successively subjected to silica gel gravity column chromatography and normal phase HPLC purification to afford compounds **1**—**7**.

Chabrolidione A (**1**) was obtained as a colorless oil. On the basis of its HR-EI-MS (*m*/*z* 236.1773, M^+) and ^{1}H- and ^{13}C-NMR spectral data, the molecular formula of **1** was established as $C_{15}H_{24}O_2$. The existence of the ketone functional group (ν_{max} 1712 cm^{-1}) in **1** was observed from IR spectrum. Inspection of the ^{13}C-NMR and DEPT spectral data (Table 1) of **1**, indicated the presence of 15 carbon signals of a sesquiterpenoid. These signals were ascribable to carbons of three methyls, five sp^3 methylenes, one sp^2 methylene, and three sp^3 methines. The remaining three signals appearing in the lower field region of the spectrum are due to the quaternary carbons of one olefinic carbon (δ 149.0) and two ketone carbonyls (δ 208.6, 214.3). By the assistance of extensive 2D NMR study (COSY, HMQC, HMBC), the 4,5-seco-guaiane skeleton[6] of **1** was proposed (Fig. 1). The relative stereochemistry of **1** was confirmed to be 1*S**, 7*S**, 10*S** from the following NOESY correlations (Fig. 2): H-10, H-8α (δ 1.82), and H-6α (δ 2.42) with H-1; H-7 with H-6β (δ 2.62); H_3-14 with H-9β (1.78). It was found that **1** has been obtained previously by chemical reaction.[7] However, this compound was discovered for the first time from natural sources.

The new metabolite chabrolidione B (**2**) was obtained as a colorless oil. A molecular formula of $C_{15}H_{24}O_3$ (*m*/*z* 275.1623, $[M+Na]^+$) for **2** was established from HR-ESI-MS data, and thus requiring four degrees of unsaturation.

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Table 1. ^{1}H- and ^{13}C-NMR Chemical Shifts for Compounds **1** and **2**

C/H	**1**		**2**	
	$^{1}H^{a)}$	$^{13}C^{b)}$	$^{1}H^{a)}$	$^{13}C^{b)}$
1	2.60 m	55.8 (CH)$^{d)}$	1.38 m; 1.55 m	35.8 (CH_2)
2	1.67 m; 1.94 m	20.8 (CH_2)	1.51 m	18.3 (CH_2)
3	2.38 m; 2.52 m	42.2 (CH_2)	2.42 m$^{e)}$	44.0 (CH_2)
4		208.6 (C)		208.9 (C)
5		214.3 (C)		204.7 (C)
6	H_α 2.42 m; H_β 2.62 m	48.8 (CH_2)	6.04 s	121.3 (CH)
7	2.34 m	43.3 (CH)		168.2 (C)
8	H_α 1.82 m; H_β 1.56 m	31.7 (CH_2)	2.42 m$^{e)}$	22.5 (CH_2)
9	H_α 1.34 m; H_β 1.78 m	33.0 (CH_2)	H_α 1.97 m; H_β 1.77 m;	33.4 (CH_2)
10	2.03 m	33.6 (CH)		43.5 (C)
11		149.0 (C)		72.6 (C)
12	1.71 s	20.2 (CH_3)	1.41 s$^{e)}$	28.6 (CH_3)
13	4.70 s; 4.71 s	109.7 (CH_2)	1.41 s$^{e)}$	28.7 (CH_3)
14	0.91 d (7.0)$^{c)}$	18.3 (CH_3)	1.07 s	21.8 (CH_3)
15	2.12 s	29.9 (CH_3)	2.13 s	29.9 (CH_3)

a) Spectra recorded at 500 MHz in $CDCl_3$. *b*) 125 MHz in $CDCl_3$. *c*) *J* values (in Hz) parentheses. *d*) Deduced from DEPT. *e*) Overlapping of signals was observed.

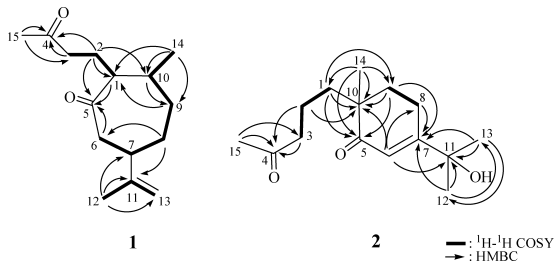


Fig. 1. Key ^{1}H–^{1}H COSY and HMBC Correlations for **1** and **2**

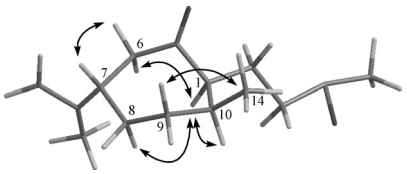


Fig. 2. Key NOESY Correlations of **1**

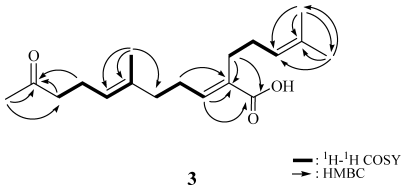
The IR spectrum of **2** suggested the presence of the hydroxy, ketone, and α,β-conjugated ketone functionalities (ν_{max} 3422, 1716, 1684 cm^{-1}, respectively). By the analysis of ^{13}C and DEPT spectra, the carbons signals were assigned into four methyls, five methylenes, one olefinic methine (δ 121.3), and five quaternary carbons including an oxygenated one (δ 72.6, s). The quaternary carbon signals appearing at δ 208.9, and 204.7 were attributable to a normal ketone and an α,β-conjugated ketone, respectively. From the ^{1}H–^{1}H COSY spectrum of **2** (Fig. 1), it was possible to establish the two proton sequences from H-1 to H-3 and H-8 to H-9. The molecular framework of **2** was further established by an HMBC experiment which showed the following key correlations (Fig. 1): H_2-1 to C-5, C-9, and C-10, H_2-2 to C-4 and C-10, H_2-3 to C-4, H-6 to C-8, C-10, and C-11, H_2-8 to C-7, H_2-9 to C-5, C-7, and C-10, both H_3-12 and H_3-13 to C-7 and C-11, H_3-14 to C-1, C-5, C-9, and C-10, and H_3-15 to C-3 and C-4. On the basis of the above findings the 4,5-secoeudesmane skeleton[8] of **2** could be established unambiguously. Furthermore, metabolite **2** ($[\alpha]_D$ −9.3°) has the same sign of optical rotation with that of a synthetic compound **8** ($[\alpha]_D$ −4.6°).[9] Thus, the absolute configuration of **2** was assumed to be 10*R*. On the basis of above analysis, the structure of **2** was established.

Table 2. ^{1}H- and ^{13}C-NMR Data for Compounds **3** and **4**

C/H	**3**		**4**	
	$^{1}H^{a)}$	$^{13}C^{b)}$	$^{1}H^{a)}$	$^{13}C^{b)}$
1	2.14 s	30.0 (CH_3)$^{d)}$	2.14 s	29.9 (CH_3)
2		208.7 (C)		208.8 (C)
3	2.47 t (7.5)$^{c)}$	43.6 (CH_2)	2.46 t (7.5)	43.7 (CH_2)
4	2.27 m	22.3 (CH_2)	2.28 m	22.4 (CH_2)
5	5.12 t (7.5)	123.7 (CH)	5.10 br s	123.3 (CH)
6		135.1 (C)		135.5 (C)
7	2.11 m	38.4 (CH_2)	2.08 t (7.5)	39.0 (CH_2)
8	2.31 m	27.2 (CH_2)	2.59 q (7.5)	28.1 (CH_2)
9	6.85 t (7.5)	145.0 (CH)	5.97 t (7.5)	144.9 (CH)
10		131.2 (C)		130.4 (C)
11	2.33 m	26.8 (CH_2)	2.28 m	34.6 (CH_2)
12	2.10 m	27.6 (CH_2)	2.12 m	27.8 (CH_2)
13	5.13 t (7.0)	123.5 (CH)	5.10 br s	123.4 (CH)
14		132.4 (C)		132.3 (C)
15	1.68 s	25.7 (CH_3)	1.69 s	25.7 (CH_3)
16	1.60 s	17.6 (CH_3)	1.59 s	17.7 (CH_3)
17		172.1 (C)		170.7 (C)
18	1.64 s	15.9 (CH_3)	1.62 s	15.9 (CH_3)

a) Spectra recorded at 500 MHz in $CDCl_3$. *b*) 125 MHz in $CDCl_3$. *c*) *J* values (in Hz) parentheses. *d*) Deduced from DEPT.

Ketochabrolic acid (**3**) was obtained as an optically inactive yellow oil. The HR-ESI-MS of **3** established the molecular formula $C_{18}H_{28}O_3$, implying five degrees of unsaturation. The ^{13}C-NMR and DEPT spectra (Table 2) of **3** showed signals of four methyls, six methylenes, three methines, and five sp^2 quaternary carbons including two carbon of a carboxylic acid (δ 172.1) and a ketone (δ 208.7), respectively. From the ^{1}H-NMR (Table 2) spectrum of **3**, the presences of an acetyl methyl as a singlet at δ_H 2.14 was revealed. The structure of **3** and all of the ^{1}H- and ^{13}C-NMR spectral data were assigned by the assistance of 2D NMR (^{1}H–^{1}H COSY and HMBC) experiments (Fig. 3). Furthermore, comparison of the NMR data between **3** and the known compound **9**[1] confirmed both compound have the same partial structure from C-3 to C-17 of **3** and from C-4 to C-18 of **9**. Hence, the full



3

Fig. 3. Key ^{1}H–^{1}H COSY and HMBC Correlations for **3**

Table 3. ^{1}H- and ^{13}C-NMR Data for Compound **5**

C/H	**5**	
	^{1}H[a]	^{13}C[b]
1′		185.1 (C)[d]
2′		130.4 (C)
3′		132.2 (C)
4′		186.0 (C)
5′		148.0 (C)
6′	6.80 d (1.2)[c]	135.7 (CH)[e]
7′	2.18 d (1.2)	16.6 (CH_3)
1	7.95 d (7.8)	126.3 (CH)
2	7.52 d (7.8)	134.0 (CH)
3		148.9 (C)
4	2.77 t (7.8)	36.2 (CH_2)
5	2.37 m	29.4 (CH_2)
6	5.16 t (8.1)	123.5 (CH)
7		135.7 (C)[e]
8	2.09 m	38.6 (CH_2)
9	2.30 m	27.2 (CH_2)
10	6.70 t (7.5)	142.5 (CH)
11		131.9 (C)
12	2.26 m	27.1 (CH_2)
13	2.09 m	27.8 (CH_2)
14	5.11 t (8.1)	123.7 (CH)
15		132.3 (C)
16	1.67 s	25.8 (CH_3)
17	1.58 s	17.7 (CH_3)
18		168.5 (C)
19	1.54 s	16.1 (CH_3)
20	7.90 d (1.2)	126.5 (CH)
COOMe	3.73 s	51.7 (CH_3)

a) Spectra recorded at 300 MHz in $CDCl_3$. *b*) 75 MHz in $CDCl_3$. *c*) *J* values (in Hz) parentheses. d) Deduced from DEPT. *e*) Overlapping of signals was observed.

structure for **3** can be determined as shown in formula **3**. HR-ESI-MS and NMR spectral data indicated that isoketochabrolic acid (**4**) has the same molecular formula, $C_{18}H_{28}O_3$, as that of **3** (Table 2). The ^{1}H-NMR spectral data of **4** were found to be similar to those of **3**, except that the signal of H-9 of **4** (δ 5.97) was shifted significantly to upper field in comparison with that of **3** (δ 6.85), and the methylene protons H_2-8 (δ 2.59) were found to show downfield shifted resonance in comparison with that of **3** (δ 2.31). Thus, **4** was found to be the 9*Z* isomer of **3**.

Chabrolonaphthoquinone C (**5**) was isolated as a yellow oil. Its molecular formula $C_{28}H_{34}O_4$ was established by HR-ESI-MS (*m/z* 457.2357, $[M+Na]^+$). Thus, twelve degrees of unsaturation were determined for the molecule of **5**. It was shown that the NMR data of **5** (Table 3) is almost identical with those of **9**,[1)] except that the carboxylic acid in **9** was replaced by a methylester (δ_H 3.73, s) in **5**. Moreover, the ^{13}C-NMR spectral data of **5** is also nearly identical with those of **9**, except for the presence of one additional methyl carbon resonating at δ_C 51.7 (CH_3). Above data establish **5** as the methyl ester of **9**.

The cytotoxicity of compounds **1**—**3** and **5**—**7**, against the proliferation of a limited panel of cancer cell lines, including human hepatocellular carcinomas (Hep G2 and Hep 3B), human breast carcinomas (MCF-7 and MDA-MB-231), and a human lung carcinoma (A-549) was studied. The results showed that **1**—**3**, **6**, and **7** are not cytotoxic toward the above cancer cells. Metabolite **5** has been shown to exhibit a moderate to weak cytotoxicity toward MDA-MB-231 (IC_{50} 8.4 μg/ml), MCF-7 (IC_{50} 11.9 μg/ml), Hep G2 (IC_{50} 16.4 μg/ml), and A-549 (IC_{50} 9.3 μg/ml) cancer cell lines.

Experimental

The IR spectra were recorded on a Jasco FT-5300 infrared spectrophotometer. UV 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 ^{1}H and 75 MHz for ^{13}C or on a Varian Unity INOVA 500 FT-NMR at 500 MHz for ^{1}H 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. HR-MS were recorded on ESI or EI-MS 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 the 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, located in 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 Biotechnology and Resources, National Sun Yat-sen University.

Extraction and Isolation The sliced bodies of *N. chabrolii* (1.8 kg, wet wt) were exhaustively homogenized with EtOH and filtered. 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 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 and EtOAc mixtures of increasing polarity, and finally pure EtOAc, to yield 28 fractions. Fraction 5, eluted with *n*-hexane–EtOAc (25 : 1), was further purified on silica gel using *n*-hexane–acetone (30 : 1) to yield **6** (8.0 mg) and **1** (5.1 mg). Fraction 7, eluted with *n*-hexane–EtOAc (15 : 1), was further separated by normal phase HPLC using *n*-hexane–acetone (20 : 1) to afford **5** (3.0 mg). Fraction 15, eluted with *n*-hexane–EtOAc (4 : 1), was purified by normal phase HPLC using *n*-hexane–acetone (8 : 1) to afford **3** (4.0 mg) and **4** (1.8 mg). Fraction 21, eluted with *n*-hexane–EtOAc (1 : 1), was further purified by normal phase HPLC using *n*-hexane–acetone (4 : 1) to afford **7** (1.0 mg) and **2** (4.5 mg).

Chabrolidione A (**1**): Colorless oil; $[\alpha]_D^{25}$ −22.0° (*c*=1.8, $CHCl_3$); IR (neat) ν_{max} 2930, 1712, 1645, 1375 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 500 MHz) and ^{13}C-NMR ($CDCl_3$, 125 MHz), see Table 1; EI-MS (70 eV) *m/z* 236 (5, $[M]^+$); HR-EI-MS *mz* 236.1773 (Calcd for $C_{15}H_{24}O_2$, 236.1771).

Chabrolidione B (**2**): Colorless oil; $[\alpha]_D^{25}$ −9.3° (*c*=0.7, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 236 (4.06) nm; IR (neat) ν_{max} 3422, 2926, 1716, 1684, 1655, 1396 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 500 MHz) and ^{13}C-NMR ($CDCl_3$, 125 MHz), see Table 1; ESI-MS *m/z* 275 (100, $[M+Na]^+$); HR-ESI-MS *m/z* 275.1623 (Calcd for $C_{15}H_{24}O_3Na$, 275.1623).

Ketochabrolic acid (**3**): Colorless oil; UV (MeOH) λ_{max} (log ε) 222 nm (4.25); IR (neat) ν_{max} 3369, 2928, 1715, 1637, 1375 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 500 MHz) and ^{13}C-NMR ($CDCl_3$, 125 MHz), see Table 2; ESI-MS *m/z* 315 (100, $[M+Na]^+$); HR-ESI-MS *m/z* 315.1935 (Calcd for $C_{18}H_{28}O_3Na$, 315.1936).

Isoketochabrolic acid (**4**): Colorless oil; UV (MeOH) λ_{max} (log ε) 222 nm (4.21); IR (neat) ν_{max} 3398, 2926, 1715, 1637, 1375 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 500 MHz) and ^{13}C-NMR ($CDCl_3$, 125 MHz), see Table 2; ESI-MS *m/z* 315 (100, $[M+Na]^+$); HR-ESI-MS *m/z* 315.1935 (Calcd for $C_{18}H_{28}O_3$, 315.1936).

Chabrolonaphthoquinone C (**5**): Pale yellow oil; UV (MeOH) λ_{max} (log ε) 345 (3.71), 267 (4.23), 258 (4.28) nm; IR (neat) ν_{max} 2922, 1680, 1660, 1630, 1600, 1379 cm^{-1}; ^{1}H- ($CDCl_3$, 300 MHz) and ^{13}C- ($CDCl_3$, 75 MHz)

NMR, see Table 3; ESI-MS *m*/*z* 457 (100, $[M+Na]^+$); HR-ESI-MS *m*/*z* 457.2357 (Calcd for $C_{28}H_{34}O_4Na$, 457.2355).

Cytotoxicity Testing Cytotoxicity assays of compounds **1**—**3** and **5**—**7** were performed using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide] colorimetric method.[10)]

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