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New Terpenoids from the Soft Corals *Sinularia capillosa* and *Nephthea chabroli*

Shi-Yie Cheng,[†,||] Ki-Jhih Huang,[†] Shang-Kwei Wang,[‡] Zhi-Horng Wen,[†] Chi-Hsin Hsu,[†] Chang-Feng Dai,[§] and Chang-Yih Duh*[,†,||]

Department of Marine Biotechnology and Resources, National Sun Yat-Sen University, Kaohsiung, Taiwan, Department of Microbiology, Kaohsiung Medical University, Kaohsiung, Taiwan, Institute of Oceanography, National Taiwan University, Taipei, Taiwan, and Asia-Pacific Ocean Research Center, National Sun Yat-sen University, Kaohsiung, Taiwan

yihduh@mail.nsysu.edu.tw

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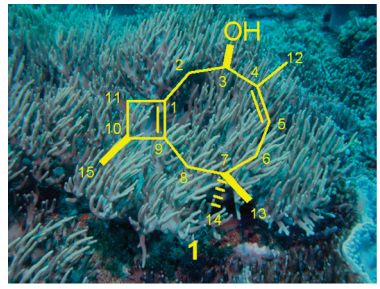
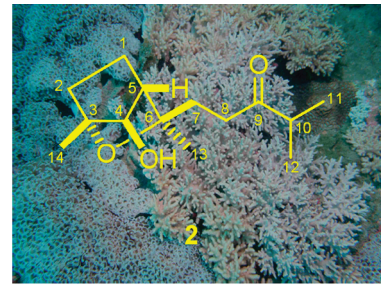
ABSTRACT



Sinularia capillosa



Nephthea chabroli

Two new terpenoids, capillosanol (1) and chabranol (2), possessing unprecedented terpenoid skeletons, were isolated from the soft corals *Sinularia capillosa* and *Nephthea chabroli*, respectively. The structures of 1 and 2 were elucidated through extensive spectroscopic analyses. The cytotoxicities of these compounds were tested in vitro.

Soft corals, especially those of the genera *Sinularia* and *Nephthea*, have been well recognized as a rich source of sesquiterpenoids, providing a wide range of structural diversity[1−12] and exhibiting various bioactivities such as cytotoxic,[3,6−9] anti-inflammatory,[9,10] and antimicrobial properties.[2,10]

As part of a continuing search for bioactive substances from marine invertebrates, we explored the chemical investigations of the Formosan soft corals *S. capillosa* Tixier-

[†] National Sun Yat-Sen University.
[‡] Kaohsiung Medical University.
[§] National Taiwan University.
[||] Asia-Pacific Ocean Research Center.

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Durivault and *N. chabroli* Audouin, which were collected from the Dongsha Atoll and Siaoliouciou Island, respectively.

Chromatographic separation on the acetone extracts of the soft corals *S. capillosa* and *N. chabroli* resulted in the isolation of two new terpenoids, named as capillosanol (**1**) and chabranol (**2**), respectively (Figure 1).

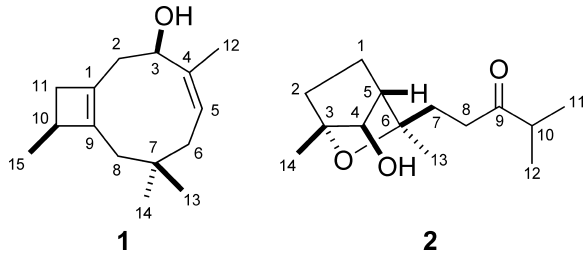

Figure 1. Structures of metabolites **1** and **2**.

The acetone extract of *S. capillosa* was concentrated to a brown gum, which was partitioned between EtOAc and H_2O. The EtOAc-soluble residue (60 g) was subjected to CC on silica gel using *n*-hexane−EtOAc mixtures of increasing polarity to yield 40 fractions. Fraction 14 (0.8 g) was applied to a C_{18} gel column to obtain a mixture (72 mg) that was further purified by HPLC (LiChrosorb RP-18, 7 μm, 25 × 250 mm), eluting with MeOH−H_2O (85:15) to yield **1** (2.0 mg). In the same manner, the EtOAc fraction (100 g) of the other soft coral *N. chabroli* was subjected to CC on silica gel to furnish 40 fractions. Fraction 16 (1.5 g) was fractionated over Sephadex LH-20 eluting with MeOH followed by RP-18 HPLC purification using MeOH−H_2O (75:25) as eluent to give **2** (1.0 mg).

Capillosanol (**1**)[13] was obtained as a white amorphous powder. The positive HRESIMS of **1** exhibited a pseudomolecular ion peak at *m/z* 243.1727 $[M + Na]^+$, consistent with the molecular formula of $C_{15}H_{24}O$, implying four degrees of unsaturation. Its IR spectrum absorptions at 3426 cm^{-1} indicated the presence of a secondary hydroxyl, which was supported by the ^1H and ^{13}C NMR signals (Table 1) resonating at δ_H 4.77 (1H, br s, H-3) and δ_C 71.0 (CH, C-3). Meanwhile, the HMBC correlations (Figure 2) observed from H_3-12 to C-3, C-4, and C-5 led to the position of the hydroxyl at C-3. The NMR spectra of **1** contained resonances for a trisubstituted double bond [δ_H 5.59 (br s, 1H); δ_C 139.5 (qC) and 122.3 (CH)] and a tetrasubstituted double bond [δ_C 130.2 (qC) and 142.5 (qC)]. The above moieties accounted for two of the four degrees of unsaturation, indicating a bicyclic structure for metabolite **1**.

From the COSY spectrum (Figure 2) of **1**, it was possible to establish the proton connects from H-3 to H_3-15 through H_2-2, H_2-11, and H-10, and from H_2-6 to H_3-12 through H-5, as well as a long-range COSY correlation between H_2-2 and

(13) Capillosaniol (**1**): white amorphous powder; $[\alpha]^{25}_D$ +114 (*c* 0.1, $CHCl_3$); IR (KBr) ν_{max} 3426, 2928, 1655, 1449, 1373 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 1; ESIMS *m/z* 243 $[M + Na]^+$; HRESIMS *m/z* 243.1727 $[M + Na]^+$ (calcd for $C_{15}H_{24}ONa$, 243.1725).

Table 1. ^1H and ^{13}C NMR Spectroscopic Data of **1**[a]

	1	
C/H	^{13}C	^1H
1	130.2 (qC)[b]	
2	34.5 (CH_2)	a: 1.76 m; b: 1.54 m
3	71.0 (CH)	4.77 br s
4	139.5 (qC)	
5	122.3 (CH)	5.59 br s
6	53.1 (CH)	a: 2.31 d (15.5);[c] b: 2.02 d (15.5)
7	37.2 (qC)	
8	54.0 (CH)	a: 2.20 d (15.0); b: 1.92 d (15.0)
9	142.5 (qC)	
10	37.9 (CH)	2.25 m
11	32.1 (CH_2)	α: 1.82 d (13.0); β: 1.40 d (13.0)
12	17.7 (CH_3)	1.68 s
13	29.5 (CH_3)	1.02 s
14	29.6 (CH_3)	1.03 s
15	23.1 (CH_3)	0.97 d (7.0)
3−OH		3.78 d (4.0)

[a] Spectra were measured in CD_3COCD_3 (^1H, 500 MHz and ^{13}C, 125 MHz). [b] Multiplicities are deduced by HSQC and DEPT experiments. [c] *J* values (in Hz) are in parentheses.

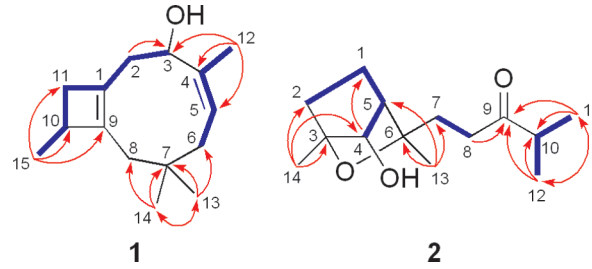

Figure 2. Selected $^1H-^1H$ COSY (—) and key HMBC (→) correlations of **1** and **2**.

H_2-11; H-5 and H_3-12 (Figure 2). The connectivities between C-9 and C-10; C-3 and C-4 were elucidated on the basis of the HMBC correlations from H_3-15 to C-9, C-10, and C-11 and from H_3-12 to C-3, C-4, and C-5. Moreover, the HMBC spectrum showed correlations from H_3-13/H_3-14 to C-6, C-7, and C-8, proving the attachment between C-6 and C-8 through C-7. Although there were no direct HMBC correlations available, the remaining one unsaturation indicated that C-8 should be linked to C-9. This assumption was further supported by the NOESY correlation from H-8a (δ_H 2.21) to H_3-15 (Figure 3). The long-range COSY correlation between H_2-2 and H_2-11 is attributed to the W-type coupling ($^4J_{2,11}$) of the highly strained ring system, which was further identified by the crucial NOESY correlation between H-11β and H-2a and absence of the NOESY correlations between H_2-2 and Me-15. Accordingly, the planar structure of metabolite **1**, possessing a bicyclo[7.2.0]undecane moiety, was proposed decidedly.

The geometry of the trisubstituted olefin was assigned as *Z* based on the NOESY correlations (Figure 3) between H-5 and H_3-12. The crucial NOE correlations between H-10 with

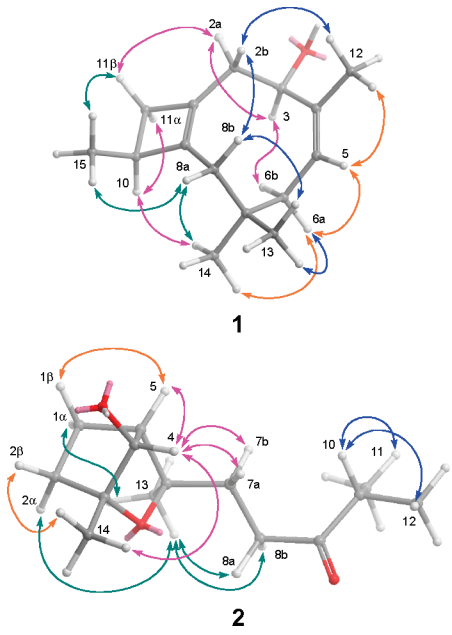

Figure 3. Key NOE correlations of **1** and **2**.

H-11α (δ_H 1.83) suggested that the two protons were oriented on the same side of the cyclobutene moiety, while H_3-15 was oriented on the opposite side. Moreover, H-2b (δ_H 1.54) was found to show NOE correlations with H-8b (δ_H 1.93) and H_3-12, and H-3 exhibited NOE correlations with H-6b (δ_H 2.00) and H-2a (δ_H 1.76), indicating the β-orientation of 3-OH. The above findings indicated the 3*R** and 10*S** configurations as depicted in Figure 3. The results, together with other detailed NOESY correlations (Figure 3) of **1**, determined the structure of capillosanol as shown in the formula **1**.

Chabranol (**2**)[14] was isolated as a colorless, viscous oil. HRESIMS of metabolite **2** exhibited a pseudomolecular ion peak at *m/z* 263.1625 $[M + Na]^+$ and established a molecular formula of $C_{14}H_{24}O_3$, indicating three degrees of unsaturation. The ^{13}C NMR (Table 1) displayed 14 carbon signals, which were identified by the assistance of the DEPT spectrum as four methyls, four methylenes, three methines, and three quaternary carbons. The ^1H NMR signal [δ_H 4.13 (br s, 1H)] (Table 2) and a broad IR absorption at 3437 cm^{-1}, together with the observation of one oxygen-bearing carbon resonance (δ_C 79.3) in ^{13}C NMR spectrum, revealed the presence of one hydroxyl. Furthermore, a keto-carbonyl carbon was recognized as being present in **2** from its ^{13}C NMR signal at δ_C 212.2 (qC, C-9), as well as from a strong IR absorption at 1714 cm^{-1}. By interpretation of COSY correlations (Figure 2), it was possible to establish three partial structures from H_2-2 to H-4 through H_2-1 and H-5 and from H_3-11 to H_3-12 through H-10, as well as COSY correlation between H_2-7 and H_2-8. The connectivities of these partial structures were further established by the HMBC correlations (Figure 2). Moreover, the HMBC correlations observed from H_2-8/H_3-11/H_3-12 to C-9 indicated the position of the keto-carbonyl group at C-9. To confirm the position of the ether linkage, **2** was submitted to acetylation with Ac_2O in pyridine at room temperature overnight. Formation of monoacetylated derivative **2a**[15] proved the presence of a secondary hydroxyl in the original structure. The HMBC correlations observed from H_3-14 to C-2/C-3/C-4 led to the assignment of the hydroxyl at C-4. Indeed, the position of the ether linkage at C-3/C-6 was confirmed by the above observations. Thus, the gross structure of **2**, possessing a cyclopentane ring fused to a tetradrofuran ring at C-3 and C-6, was elucidated unmistakably.

Table 2. ^1H and ^{13}C NMR Spectroscopic Data of **2**[a]

	2	
C/H	^{13}C	^1H
1	22.5 (CH_2)[b]	α: 1.84 m; β: 2.64 m
2	33.0 (CH_2)	α: 1.44 m; β: 1.62 m
3	84.5 (qC)	
4	79.3 (CH)	4.13 br s
5	50.2 (CH)	2.00 br s
6	78.7 (qC)	
7	36.0 (CH_2)	a: 1.91 m; b: 1.57 m
8	35.4 (CH_2)	a: 2.63 m; b: 2.35 m
9	212.2 (qC)	
10	41.7 (CH)	2.58 m
11	19.5 (CH_3)	1.09 d (7.2)[c]
12	19.3 (CH_3)	1.11 d (7.2)
13	24.0 (CH_3)	1.16 s
14	18.1 (CH_3)	1.20 s

[a] Spectra were measured in $CDCl_3$ (^1H, 400 MHz and ^{13}C, 100 MHz). [b] Multiplicities are deduced by HSQC and DEPT experiments. [c] *J* values (in Hz) are in parentheses.

The relative configuration of **2** was determined through inspection of the NOESY spectrum as well as a computer-generated lower energy conformation using MM2 force field calculations (Figure 3). From the NOESY spectrum of **2**, H-1β was found to show an NOE correlation with H-5, and H-2β exhibited an NOE correlation with H_3-14, indicating the β-orientations of H-5 and H_3-14. In addition, H-4 was determined as α on the basis of the analysis of coupling constants and splitting patterns of H-5. This finding was supported by the observation of a very small coupling constant (close to zero) between H-4 and H-5, implying the dihedral angle between the above two protons was almost 90°, consistent with the observation in the computer-modeled structure of **2**. Furthermore, the NOE correlations could be observed between H-1α/H_3-13 and H-2α/H_3-13. Thus, H_3-13 should be placed on the α face. The above findings indicated the 3*R**,4*R**,5*S**,6*R** configuration as depicted in

(14) Chabranol (**2**): colorless, viscous oil; $[\alpha]^{25}_D$ −56 (*c* 0.1, $CHCl_3$); IR (KBr) ν_{max} 3437, 2969, 2937, 1714, 1458, 1374 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 1; ESIMS *m/z* 263 $[M + Na]^+$; HRESIMS *m/z* 263.1625 $[M + Na]^+$ (calcd for $C_{14}H_{24}O_3Na$, 263.1623).

(15) 4(*R**)-Acetoxychabranol (**2a**): colorless, viscous oil; $[\alpha]^{25}_D$ −38 (*c* 0.1, $CHCl_3$); selected ^1H NMR ($CDCl_3$, 400 MHz) δ 4.92 (1H, br s, H-4), 2.63 (1H, m, H-8a), 2.39 (1H, m, H-8b), 2.60 (1H, m, H-10), 2.44 (1H, m, H-1β), 2.28 (1H, br s, H-5), 2.08 (3H, s, 4-OAc), 1.21 (3H, s, Me-14), 1.17 (3H, s, Me-13), 1.11 (3H, d, $J = 6.8$, Me-12), 1.10 (3H, $J = 6.8$, Me-11); ESIMS m/z 305.4 $[M + Na]^+$.

Figure 3. On the basis of the above observations and other detailed NOESY correlations (Figure 3), the structure of chabranol (**2**) was established unambiguously.

It is worthwhile to mention that metabolite **1** has a previously unknown carbon skeleton. We propose the name "capillosane" for this new skeleton. Farnesyl pyrophosphate may be involved in the biosynthesis of compound **1** through cyclization, oxidation, 1,3-hydrogen shift, 1,2-methyl migration, 1,2-hydrogen shift, and deprotonation to result in the formation of a capillosane-type skeleton (see the Supporting Information). A possible biosynthetic pathway for the loss of a carbon fragment from cyclopentane sesquiterpene by enzymatic oxidative modifications that could provide a cyclopentane norsesquiterpene skeleton of **2** was postulated.

Compounds **1**, **2**, and **2a** were evaluated for cytotoxicity assays against P-388 (mouse lymphocytic leukemia), A-459 (human lung carcinoma), and HT-29 (human colon adenocarcinoma) cancer cell lines. Compounds **2** and **2a** displayed moderate cytotoxicity against P-388, with an ED_{50} of 1.81 and 3.03 μg/mL, respectively. With the exception of the above findings, the obtained negative results showed that they were not cytotoxic against these cancer cell lines (ED_{50} > 50 μg/mL). The in vitro cytotoxic assays were carried out according to the procedure described previously.[16]

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Supporting Information Available: ^{1}H NMR, ^{13}C NMR, COSY, NOESY, HMQC, and HMBC spectra for **1** and **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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