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

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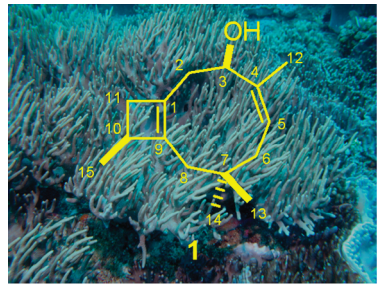
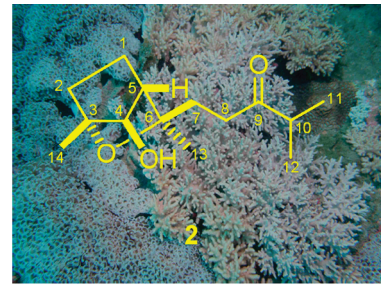
## ABSTRACT




***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-

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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).

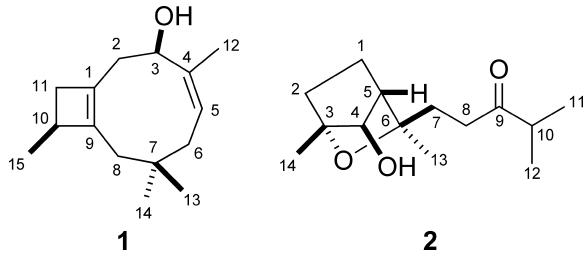


**Figure 1.** Structures of metabolites **1** and **2**.

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 $\mu$m, 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 $\delta_H$ 4.77 (1H, br s, H-3) and $\delta_C$ 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 [$\delta_H$ 5.59 (br s, 1H); $\delta_C$ 139.5 (qC) and 122.3 (CH)] and a tetrasubstituted double bond [$\delta_C$ 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) $\nu_{max}$ 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 ($CH_2$) | $\alpha$: 1.82 d (13.0); $\beta$: 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.

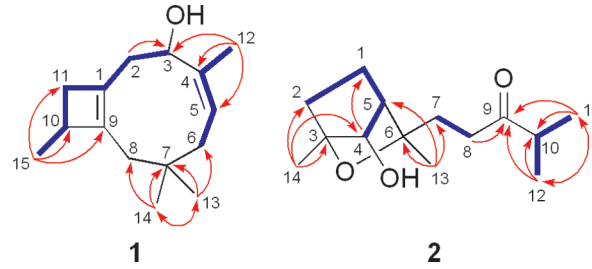


**Figure 2.** Selected $^1H-^1H$ COSY (—) and key HMBC (→) correlations of **1** and **2**.

$H_2$-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 ($\delta_H$ 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-11$\beta$ 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

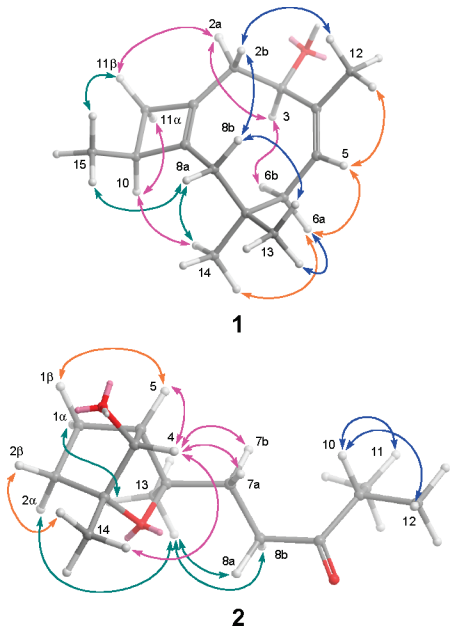


**Figure 3.** Key NOE correlations of **1** and **2**.

H-11α ($\delta_H$ 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 ($\delta_H$ 1.54) was found to show NOE correlations with H-8b ($\delta_H$ 1.93) and $H_3$-12, and H-3 exhibited NOE correlations with H-6b ($\delta_H$ 2.00) and H-2a ($\delta_H$ 1.76), indicating the $\beta$-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 [$\delta_H$ 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 ($\delta_C$ 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 $\delta_C$ 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

(14) Chabranol (**2**): colorless, viscous oil; $[\alpha]^{25}_D$ −56 (*c* 0.1, $CHCl_3$); IR (KBr) $\nu_{max}$ 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).

**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.

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.

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-1$\beta$ was found to show an NOE correlation with H-5, and H-2$\beta$ exhibited an NOE correlation with $H_3$-14, indicating the $\beta$-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

(15) 4(*R**)-Acetoxychabranol (**2a**): colorless, viscous oil; $[\alpha]^{25}_D$ −38 (*c* 0.1, $CHCl_3$); selected $^1H$ NMR ($CDCl_3$, 400 MHz) $\delta$ 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-1$\beta$), 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 $\mu$g/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 $\mu$g/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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