# Terpiodiene: A Novel Tricyclic Alcohol from the Okinawan Sponge *Terpios hoshinota*

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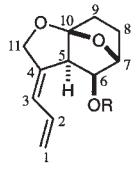
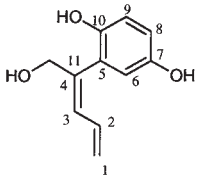
Terpiodiene (**1**), a novel tricyclic alcohol, was isolated from the Okinawan sponge *Terpios hoshinota*, which overgrows and kills corals. Its structure was determined by spectroscopic analysis and the modified Mosher's method. This compound exhibited moderate cytotoxicity against P388 cells.

Corals are important sources of revenue for the fishing and tourism industries and growing sources of medicines, including unique antimicrobial and anticancer agents,[1,2] but they are rapidly disappearing. Their decline has been attributed to several causes, including overfishing, pollution, typhoons, global warming and increased numbers of the crown-of-thorns starfish *Acanthaster planci*. Recently, the overgrowth of hard-coral communities by other organisms has been recognized as a factor that contributes to their destruction.[3] It has been reported that these organisms produce compounds that are toxic to corals. For example, the steroidal alkaloids plakinamines A and B were isolated from the marine sponge *Plakina* sp. which overgrows coral heads.[4] Nakienones A-C and nakitriol were isolated from the Okinawan cyanobacteria *Synechocystis* sp. which also overgrows coral reefs.[5] In addition, we have found that the Okinawan sponge *Terpios hoshinota* overgrows and kills corals. We report here the isolation and structural determination of a novel compound, **1**, from this unique sponge.



**1** : R = H (Terpiodiene)
**2** : R = Ac
**3** : R = (*R*)-MTPA
**4** : R = (*S*)-MTPA



Nakitriol (**5**)

The Okinawan sponge *Terpios hosinota* (5.0 kg), collected at Nakijin, Okinawa Prefecture, was extracted with 75% ethanol (8 L) for 7 days. The extract was filtered, concentrated, and partitioned between EtOAc and $H_2O$. The EtOAc-soluble material was further partitioned between 90% aqueous MeOH and hexane. The material obtained from the aqueous MeOH portion was subjected to fractionation using silica gel ($CHCl_3$-MeOH), ODS silica gel (60% aqueous MeOH to MeOH) and reversed-phase HPLC (Develosil ODS-HG-5, 30% aqueous MeOH to MeOH) to give **1** and nakitriol (**2**) as a colorless oil. Terpiodiene and nakitriol exhibited cytotoxicity against P388 cells, with $IC_{50}$ values of 18 $\mu$g/mL and 0.47 $\mu$g/mL, respectively.

The molecular formula of **1** was found to be $C_{11}H_{14}O_3$ by HRFABMS ($m/z$ 217.0842, calcd for $C_{11}H_{14}O_3Na$ $[M + Na]^+$ 217.0841). The NMR data for **1** is summarized in Table 1. The $^1H$ NMR, $^{13}C$ NMR, and HMQC spectra of **1** showed the presence of three $sp^3$-methylene carbons, three $sp^3$-methine carbons, one quaternary carbon, and four olefinic carbons ($\delta_C$ 114.3, 122.9, 135.6, 143.0). The carbon chemical shifts of **1** suggests that one methylene ($\delta_C$ 68.67) and two methine carbons ($\delta_C$ 68.70, 73.3) are connected to an oxygen atom and that the quaternary carbon ($\delta_C$ 104.8) is an acetal carbon. Since compound **1** has two carbon-carbon double bonds and no carbonyl carbon, **1** was confirmed to be tricyclic based on its molecular formula and degree of unsaturation. An analysis of $^1H$ NMR and COSY spectra of **1** allowed a conjugated diene (C1-C3), an oxymethylene group (C11) and a partial structure (C5-C9) to be constructed (Figure 1). The HMBC correlations (H3/C11, H3/C4, H5/C4, H5/C10, H9/C10) disclosed connectivity between all of the carbon atoms. The location of a hydroxyl group in **1** was determined by the downfield shifts observed for H6 ($\delta$3.18 $\rightarrow$ 4.45) in the $^1$NMR spectrum of monoacetate **2**, which was prepared by acetylation of **1**. Thus, the

**Table 1.** NMR data for **1** in $CD_3OD$

| Positoin | $^1H$ (/ppm) | $^{13}C$ (/ppm) | | HMBC ($^1H \rightarrow ^{13}C$) |
|---|---|---|---|---|
| 1a | 4.99 dd[a,c] (10.4, 0.8) | 114.3 | t[b,d] | C-3 |
| 1b | 5.08 dd (16.4, 0.8) | | | C-2, 3 |
| 2 | 6.72 ddd (16.4, 11.0, 10.4) | 135.6 | d | C-3, 4 |
| 3 | 6.07 d (11.0) | 122.9 | d | C-1, 2, 5, 11 |
| 4 | | 143.0 | s | |
| 5 | 2.92 d (9.5) | 50.4 | d | C-3, 4, 6, 7, 10, 11 |
| 6 | 3.18 dd (9.5, 2.4) | 73.3 | d | C-4, 5, 7 |
| 7 | 3.87 ddd (4.8, 2.4, 2.4) | 68.70 | d | C-5, 6, 9 |
| 8a | 1.59 dddd (14.1, 14.1, 4.8, 2.4) | 26.5 | t | C-6, 7, 9, 10 |
| 8b | 1.74 dddd (14.1, 4.8, 4.8, 2.2) | | | C-9, 10 |
| 9a | 2.07 ddd (14.1, 14.1, 4.8) | 27.4 | t | C-5, 7, 8, 10 |
| 9b | 1.81 ddd (14.1, 4.8, 2.2) | | | C-7, 8, 10 |
| 10 | | 104.8 | s | |
| 11a | 4.47 d (13.4) | 68.67 | t | C-3, 4, 5 |
| 11b | 4.50 d (13.4) | | | C-3, 4, 5 |

[a]Recorded at 800 MHz. [b]Recorded at 200 MHz. [c]Coupling constants (Hz) are in parentheses. Signal of hydroxy group was not observed. [d]Multiplicity was based on the HMQC spectrum.

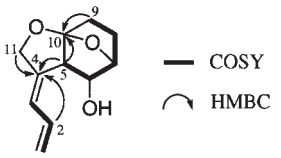



**Figure 1.** Partial structures of **1**, based on 2D NMR correlations.

gross structure of **1** was determined to be as shown in Figure 1.

The relative stereochemistry of **1** was determined as follows: A plausible conformation of **1** with the important NOESY correlations is shown in Figure 2. The vicinal spin-spin coupling constants ($J_{5,6} = 9.5$ Hz, $J_{6,7} = 2.4$ Hz, $J_{7,8a} = 2.4$ Hz, $J_{8a,9a} = 14.1$ Hz) and the NOESY correlations (H5/H6, H6/H8, H5/H9) indicated that the **1** had a rigid conformation and that the orientations of H5, H6, and H7 were axial, axial, and equatorial, respectively (Figure 2). The geometry of the C3 olefin was also determined to be 3*E* based on the NOESY correlation between H-3 and H-11. Thus, the relative stereochemistry of **1** was determined to be as shown in Figure 2.

The absolute stereochemistry of C6 was determined using the

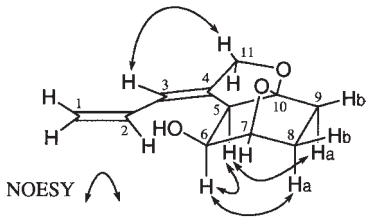



**Figure 2.** Relative stereochemistry of **1**, based on NOESY correlations.

modified Mosher's method.[6] The $^1$H NMR signals of the two MTPA esters, **3** and **4**, were assigned based on the 2D NMR spectra, and the $\Delta\delta$ values ($\delta_S$-$\delta_R$, ppm) were then calculated. The results (Figure 3) established that the absolute stereochemistry of C6 is *S*. Therefore, the absolute stereochemistry of **1** was determined to be 5*R*, 6*S*, 7*R* and 10*S*.

The analytical properties ($^1$N NMR, $^{13}$C NMR) of **5** were identical in all respects to those in the literature.[5] We found that **5** also exhibited strong antibacterial activity against *Rhodospirillium Salexigens* SCRC 113 (25 mm, 0.1 mg per disk) in addition to its cytotoxic effects. This gram-negative bacteria shows adhering properties.[7]

In conclusion, terpiodiene, a novel tricyclic alcohol, and nakitriol were isolated from the Okinawan sponge *Terpios*

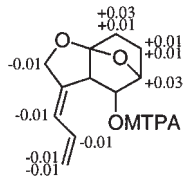



**Figure 3.** $\Delta\delta$ values ($\delta_S$-$\delta_R$) for the MTPA esters **3** and **4** in ppm.

*hoshinota*. The structure of terpiodiene was determined by its 2D NMR spectra and the modified Mosher's method.

Terpiodiene and nakitriol have similar frameworks, which implies that these compounds may be formed by similar biosynthetic processes. Terpiodiene is considered to be a biosynthetic intermediate of nakitriol. Since terpiodiene and nakitriol each exhibit moderate cytotoxicity against P388 cells, the role of these cytotoxic compounds in the relationship between this sponge and corals is of interest.

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