(–)-Hydroxylindestrenolide, a New Sesquiterpenoid from a Gorgonian Coral *Menella* sp. (Plexauridae)

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(–)-Hydroxylindestrenolide (1), an enantiomer of the known sesquiterpenoid, (+)-hydroxylindestrenolide (2), was isolated from a gorgonian coral identified as *Menella* sp. The structure, including the relative stereochemistry of 1 was established by spectroscopic methods and by comparison the spectral and physical data with those of 2. Sesquiterpenoid 1 exhibited marginal cytotoxicity toward HL-60 and K562 leukemia tumor cells, and displayed a weak inhibitory effect on superoxide anion generation at a concentration of 10 μ g/ml by human neutrophils.

Key words *Menella*; sesquiterpenoid; gorgonian; hydroxylindestrenolide; enantiomer; cytotoxicity

In previous studies, a series of interesting secondary metabolites including steroids,^{1–3)} guaiane lactones,^{4,5)} briarane diterpenoids,³⁾ menellin A (a highly oxygenated race-mate with C₈ skeleton),³⁾ picolinic acid *N*-methyl betaine,^{1,6)} *n*-hexadecanol,¹⁾ 9*H*-purin-6-amino-*N*-9-dimethyl,¹⁾ thymidine,¹⁾ and batyl alcohol,^{1,7)} had been reported from the gorgonian corals belonging to the genus *Menella*, collected off the South China Sea. In a continuation of our search for bioactive substances from marine invertebrates, a specimen of gorgonian coral identified as *Menella* sp., collected off southern Taiwan coast, was studied. Its organic extract exhibited cytotoxicity toward HL-60 (human promyelocytic leukemia) tumor cells (IC₅₀ = 27.6 μ g/ml) and displayed significant inhibitory effects on superoxide anion generation (inhibition rate 84.7%) and elastase release (inhibition rate 96.2%) at a concentration of 10 μ g/ml by human neutrophils, respectively. A new sesquiterpenoid, (–)-hydroxylindestrenolide (**1**) (Chart 1), was isolated from this organism. In this paper, we report the isolation, structure determination, and bioactivity of this new sesquiterpenoid.

Results and Discussion

(–)-Hydroxylindestrenolide (**1**) was isolated as a white powder, and the molecular formula of this compound was determined to be C₁₅H₁₈O₃ (seven degrees of unsaturation) from a pseudomolecular ion at *m/z* 247 in the electrospray ionization (ESI)-MS spectrum and further supported by high resolution (HR)-ESI-MS (*m/z* 247.1329, Calcd 247.1329, [C₁₅H₁₈O₃ + H]⁺). Comparison of the ¹³C-NMR and distortionless enhancement by polarization transfer (DEPT) spectral data with the molecular formula indicated that there must be an exchangeable proton, requiring the presence of a hydroxy group in **1**, and this deduction was supported by a

broad absorption in the IR spectrum at 3363 cm^{–1}. The IR absorption at 1743 cm^{–1} also showed the presence of α,β -unsaturated γ -lactone group in **1**. From the ¹H- and ¹³C-NMR spectra (Table 1), **1** was found to possess a γ -lactone moiety (δ_C 172.4, s, C-12), a tetrasubstituted olefin (δ_C 161.0, s, C-7; 122.3, s, C-11), a disubstituted olefin (δ_H 5.55, 1H, d, *J* = 10.0 Hz, H-1; 5.48, 1H, ddd, *J* = 10.0, 3.2, 3.2 Hz, H-2; δ_C 137.5, d, C-1; 123.0, d, C-2), and an exocyclic carbon–carbon double bond (δ_H 4.98, 1H, s, H-15a; 4.76, 1H, s, H-15b; δ_C 145.3, s, C-4; 107.5, t, C-15). From the above data, four degrees of unsaturation were accounted for and **1** must be tricyclic. In the ¹H-NMR spectrum of **1**, a methyl signal (δ_H 1.09, 3H, s, H₃-14), a vinyl methyl (δ_H 1.81, 3H, d, *J* = 1.2 Hz, H₃-13), three pairs of *sp*³ methylene protons (δ_H 2.87, 1H, m; 2.82, 1H, m, H₂-3; 2.71, 1H, dd, *J* = 13.2, 2.8 Hz; 2.52, 1H, ddd, *J* = 13.2, 13.2, 1.2 Hz, H₂-6; 2.34, 1H, d, *J* = 13.6 Hz; 1.67, 1H, d, *J* = 13.6 Hz, H₂-9), and an aliphatic methine proton (δ_H 2.14, 1H, dd, *J* = 13.2, 2.8 Hz, H-5), were observed.

From the ¹H–¹H correlation spectroscopy (COSY) spectrum of **1**, it was possible to identify the separate spin systems among H-1/H-2/H₂-3; H₂-3/H₂-15 (by allylic coupling); H-5/H₂-6; and H-5/H₂-15 (by allylic coupling) (Table 1). It is noted that a ⁵*J*-long range ¹H–¹H coupling between one pro-

(–)-Hydroxylindestrenolide (**1**) (+)-Hydroxylindestrenolide (**2**)

Chart 1

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Table 1. ^1H - and ^{13}C -NMR Data, ^1H - ^1H COSY, and HMBC Correlations for Sesquiterpenoid **1**

C/H	$\delta_{\text{H}}^{\text{a)}$	$\delta_{\text{C}}^{\text{b)}$	^1H - ^1H COSY	HMBC (H $\rightarrow$ C)
1	5.55 d (10.0) ^{c)}	137.5 (d) ^{d)}	H-2, H ₂ -3	C-9, -10
2	5.48 ddd (10.0, 3.2, 3.2)	123.0 (d)	H-1, H ₂ -3	C-3, -10
3 α	2.82 m	34.8 (t)	H-1, H-2, H-3 β , H ₂ -15	C-4
3 β	2.87 m		H-1, H-2, H-3 α , H ₂ -15	C-4
4		145.3 (s)		
5	2.14 dd (13.2, 2.8)	49.9 (d)	H ₂ -6, H ₂ -15	n.o. ^{e)}
6 α	2.52 ddd (13.2, 13.2, 1.2)	24.0 (t)	H-5, H-6 β , H ₃ -13	C-5, -7, -8, -10, -11
6 β	2.71 dd (13.2, 2.8)		H-5, H-6 α	C-5, -7, -10, -11
7		161.0 (s)		
8		103.6 (s)		
9 α	2.34 d (13.6)	48.3 (t)	H-9 β	C-1, -8, -10, -14
9 β	1.67 d (13.6)		H-9 α	C-1, -5, -7, -8, -10, -14
10		37.6 (s)		
11		122.3 (s)		
12		172.4 (s)		
13	1.81 d (1.2)	8.2 (q)	H-6 α	C-7, -11, -12
14	1.09 s	20.3 (q)		C-1, -5, -9, -10
15a	4.98 s	107.5 (t)	H ₂ -3, H-5, H-15b	C-3, -5
b	4.76 s		H ₂ -3, H-5, H-15a	C-3, -5

a) Spectra measured at 400 MHz in CDCl_3 at 25 °C. b) Spectra measured at 100 MHz in CDCl_3 at 25 °C. c) J values (in hertz) in parentheses. d) Multiplicity deduced by DEPT and HMQC experiments and indicated by usual symbols. e) n.o.=not observed.

ton of C-6 methylene (δ_{H} 2.52) and H₃-13 (δ_{H} 1.81) was detected ($J=1.2$ Hz). These data, together with the assistance of heteronuclear multiple-bond coherence (HMBC) correlations between H-1/C-9, -10; H-2/C-3, -10; H₂-3/C-4; H₂-6/C-5, -7, -8, -10, -11; and H₂-9/C-1, -5, -7, -8, -10, -14, established the main carbon skeleton of **1** (Table 1). An exocyclic carbon-carbon double bond at C-4 was confirmed by the HMBC correlations between H₂-15/C-3, -5, and further supported by the allylic coupling between H₂-3/H₂-15 and H-5/H₂-15. The vinyl methyl group at C-11 was established by the HMBC correlations between H₃-13/C-7, -11, -12. The ring junction C-14 methyl was positioned at C-10 from the HMBC correlations between H₃-14/C-1, -5, -9, -10 and H₂-9/C-14. Thus, the remaining hydroxy group should be positioned at C-8 and be a part of a hemiketal constellation on the basis of a characteristic carbon signal at δ_{C} 103.6 (s, C-8). Therefore, the planar structure of **1** was established.

The relative configuration of **1** was elucidated by a nuclear Overhauser effect spectroscopy (NOESY) spectrum was compatible with those of **1** offered by computer modeling (Table 2), in which the close contacts of atoms calculated in space were consistent with the NOESY correlations. In the NOESY experiment of **1**, H-5 (δ_{H} 2.14) showed correlations with one protons of C-3 (δ_{H} 2.87), C-6 (δ_{H} 2.71), and C-9 (δ_{H} 1.67) methylenes, respectively, indicating that these protons were situated on the same face and assigned as β protons. Furthermore, C-14 methyl showed correlations with H-6 α (δ_{H} 2.52) and H-9 α (δ_{H} 2.34), but not with H-5, suggesting that C-14 methyl was α -oriented at C-10. The coupling constants of H-5 and H-6 α / β ($J=13.2$, 2.8 Hz) suggested that both H-5 and H-6 α were axial hydrogens. These results further supported the *trans* relationship between H-5 and C-14 methyl. The *cis* geometry of C-1/2 double bond was indicated by a 10.0 Hz coupling constant between H-1 (δ_{H} 5.55) and H-2 (δ_{H} 5.48), and further supported by a correlation between these two protons. However, due to the signal of hydroxy proton was not observed in the ^1H -NMR spectrum of **1**, so the stereochemistry of C-8 hydroxy group cannot be de-

Table 2. The Stereoview of **1** (Generated from Computer Modeling) and the Calculated Distances (Å) between Selected Protons Having Key NOESY Correlations

(-)-Hydroxylindestrenolide (1)	H/H	(Å)
H-1/H-2	2.42
	H-3 β /H-5	2.74
	H-5/H-6 β	2.45
	H-5/H-9 β	2.56
	H-6 α /H ₃ -14	2.21
	H-9 α /H ₃ -14	2.52

termined by this way.

The spectral (^{13}C -NMR and IR) and physical (melting point) data of **1** were in full agreement with those of a known sesquiterpenoid, (+)-hydroxylindestrenolide (**2**),⁸⁻¹⁰⁾ (Chart 1) which was first isolated from the roots of a traditional Chinese and Japan medicinal using herb plant, *Lindera strychnifolia*,⁹⁾ and this compound also had been obtained from an Indian plant *Commiphora myrrha*.¹¹⁾ However, the optical rotation value of **1** ($[\alpha]_{\text{D}}^{22} -218^\circ$ ($c=0.5$, CHCl_3)) was substantially different from that of **2** ($[\alpha]_{\text{D}} +209.8^\circ$ ($c=1.003$)⁹⁾; $[\alpha]_{\text{D}}^{25} +202.5^\circ$ ($c=1.032$)¹⁰⁾), indicating that sesquiterpenoid **1** is an enantiomer of **2**. Based on these findings, the C-8 hydroxy group in **1** should be α -oriented by comparison the structure of **1** with that of **2**. Chemical investigations of the gorgonian corals belonging to the genus *Menella* have afforded a diverse range of secondary metabolites,¹⁻⁷⁾ however, the sesquiterpenoids possessing the carbon skeleton as presented as **1** are rarely found in marine organisms.¹²⁾

In bioactivity testing, sesquiterpenoid **1** was found to exhibit marginal cytotoxicity toward HL-60 human promyelocytic leukemia and K562 human erythromyeloblastoid leukemia tumor cells ($\text{IC}_{50}=15.6$, 38.7 $\mu\text{g/ml}$), respectively, but not active toward DU-145 human prostate tumor cells ($\text{IC}_{50}>40$ $\mu\text{g/ml}$). The *in vitro* anti-inflammatory effects of **1**

were tested. Sesquiterpenoid **1** displayed a weak inhibitory effect on superoxide anion generation (inhibition rate 13.4%), but not active in inhibition of elastase release at a concentration of 10 $\mu\text{g}/\text{ml}$ by human neutrophils. The other potential components from this organism will be studied in the future.

Experimental

General Melting points were determined using a Fargo apparatus and were uncorrected. Optical rotation values were measured with a Jasco P-1010 digital polarimeter. Infrared spectra were obtained on a Varian Digilab FTS 1000 Fourier transform-IR (FT-IR) spectrophotometer. NMR spectra were recorded on a Varian Mercury Plus 400 FT-NMR at 400 MHz for ^1H -NMR and 100 MHz for ^{13}C -NMR, respectively, in CDCl_3 . Proton chemical shifts were referenced to the residual CHCl_3 signal (δ_{H} 7.26 ppm). ^{13}C -NMR spectra were referenced to the center peak of CDCl_3 at δ_{C} 77.1 ppm. ESI-MS data were recorded on a Thermo Finnigan LCQ ion-trap mass spectrometer. HR-ESI-MS data were recorded on a Thermo Fisher Scientific LTQ Orbitrap XL mass spectrometer. Column chromatography was performed on silica gel (230–400 mesh, Merck, Darmstadt, Germany). TLC was carried out on precoated Kieselgel 60 F_{254} (0.25 mm, Merck) and spots were visualized by spraying with 10% H_2SO_4 solution followed by heating. HPLC was performed using a system comprised of a Hitachi L-7100 pump, a Hitachi L-7455 photodiode array detector, and a Rheodyne injection port. A normal phase column (Hibar 250 $\times$ 10 mm, Merck, silica gel 60, 5 μm) was used for HPLC.

Animal Material Specimens of the gorgonian coral *Menella* sp. were collected by trawling off the coast of southern Taiwan in December 2004 at a depth of 100 m. This organism was identified by comparison with previous descriptions.¹³⁾ A voucher specimen (NMMBA-TW-GC-005) was deposited in the National Museum of Marine Biology and Aquarium, Taiwan.

Extraction and Isolation The freeze-dried and minced material of *Menella* sp. (wet weight 451 g, dry weight 411 g) was extracted with ethyl acetate (EtOAc) at room temperature. The EtOAc layer was separated on silica gel and eluted using the mixtures of *n*-hexane/EtOAc (stepwise) to yield fractions 1–16. Fraction 6 was separated by normal phase high performance liquid chromatography (NP-HPLC), using the mixtures of *n*-hexane and EtOAc to yield the fractions 6A–6P. Fraction 6C was further purified by NP-HPLC using the mixtures of *n*-hexane and EtOAc (6:1) to afford **1** (12.9 mg).

(–)-Hydroxylindestrenolide (**1**): White powder; mp 203–205 °C; $[\alpha]_{\text{D}}^{22}$ –218° ($c=0.5$, CHCl_3); IR (neat) ν_{max} 3363, 1743 cm^{-1} ; ^1H -NMR (CDCl_3 , 400 MHz) and ^{13}C -NMR (CDCl_3 , 100 MHz) NMR data: see Table 1. ESI-MS m/z : 247 ($\text{M}+\text{H}$)⁺; HR-ESI-MS m/z : 247.1329 (Calcd for $\text{C}_{15}\text{H}_{18}\text{O}_3+\text{H}$, 247.1329).

Molecular Mechanics Calculations Implementation of the MM2 force field¹⁴⁾ in CHEM3D PRO software from CambridgeSoft Corp., Cambridge, MA, U.S.A. (ver 9.0, 2005), was used to calculate molecular models.

Cytotoxicity Testing The cytotoxicity of compound **1** was assayed with a modification of the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric method. Cytotoxicity assays was carried out according to the procedures described previously.^{15,16)}

Human Neutrophil Superoxide Anion Generation and Elastase Release Human neutrophils were obtained by means of dextran sedimentation and Ficoll centrifugation. Superoxide anion generation and elastase release were carried out according to the procedures described previously.^{17,18)}

Briefly, superoxide anion production was assayed by monitoring the superoxide dismutase-inhibitable reduction of ferricytochrome *c*. Elastase release experiments were performed using MeO-Suc-Ala-Ala-Pro-Valp-nitroanilide as the elastase substrate.

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