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CURCUPHENOL DERIVATIVES FROM THE GORGONIAN *ECHINOMURICEA* SP.

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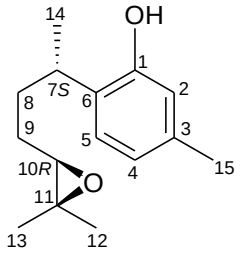
Abstract – Two sesquiterpenoid phenols, including a new natural product **1** [(7*S*, 10*R*)-(+)-10,11-epoxycurcuphenol] and a known metabolite, (+)-curcuphenol (**2**), were isolated from a gorgonian coral identified as *Echinomuricea* sp. The structures of **1** and **2** were elucidated by interpretations of spectral data and by comparison of the related physical and spectral data with those of related metabolites. Compound **2** was found to show significant inhibitory effects on elastase release by human neutrophils.

In the interest of identifying new substances from Taiwanese marine invertebrates, we studied the Taiwanese gorgonian coral *Echinomuricea* sp. for its organic extract showed interesting chemical constituents by NMR data analysis. In this paper, we report the isolation, structure determination, and bioactivity of two sesquiterpenoid phenols, including a new natural product **1** [(7*S*,10*R*)-(+)-10,11-epoxycurcuphenol] and a known substance, (+)-curcuphenol (**2**), from the studies on *Echinomuricea* sp. The structure of new natural product **1** was established by spectroscopic methods.



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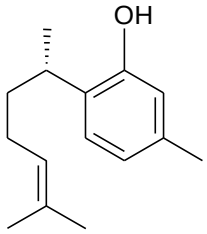

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Sesquiterpenoid phenol **1** was obtained as a yellowish oil. The molecular formula of **1** was established as $C_{15}H_{22}O_2$ (five degrees of unsaturation) from a sodiated molecule at *m/z* 257 in the ESIMS spectrum and was supported by HRESIMS (*m/z* calcd: 257.1517; found: 257.1516, $[C_{15}H_{22}O_2+Na]^+$). The IR spectrum of **1** showed a broad band at 3362 cm^{-1}, consistent with the presence of hydroxy group. From the ^1H and ^{13}C NMR data (Table 1), an 1,2,4-trisubstituted beneze ring (δ_H 6.67, 1H, s, H-2; 6.72, 1H, d, *J*=8.0 Hz, H-4; 7.01, 1H, d, *J*=8.0 Hz, H-5; δ_C 153.6, s, C-1; 137.2, s, C-3; 129.3, s, C-6; 126.4, d, CH-5; 121.7, d, CH-4; 117.8, d, CH-2). A trisubstituted epoxide containing two methyl substituents was elucidated from the signals of a quaternary oxygenated carbon at δ_C 59.0 (s, C-11), an oxymethine at δ_C 66.4 (d, CH-10), a proton signal at δ_H 2.85 (1H, dd, *J*=9.6, 2.4 Hz, H-10), and two methyl singlets at δ_H 1.33 and 1.22 (each 3H×s, H_3-12 and H_3-13). In addition, a secondary methyl (δ_H 1.25, 3H, d, *J*=6.8 Hz, H_3-14), an olefinic methyl (δ_H 2.26, 3H, s, H_3-15), two pairs of aliphatic methylene protons (δ_H 1.86, 1H, m; 1.74, 1H, m, H_2-8; 1.69, 1H, m; 1.14, 1H, m, H_2-9), an aliphatic methine proton (δ_H 3.17, 1H, m, H-7), and a hydroxy proton (δ_H 6.79, 1H, s, OH-1) were observed in the ^1H NMR spectrum of **1**.

Table 1. ^1H and ^{13}C NMR Chemical Shifts and HMBC and ^1H–^1H COSY Correlations for **1**

C/H	^1H[a]	^{13}C[b]	HMBC (H→C)	^1H–^1H COSY
1		153.6 (s)[d]		
2	6.67 (1H, s)	117.8 (d)	C-1, -4, -6	H-4
3		137.2 (s)		
4	6.72 (1H, d, *J*=8.0 Hz)[c]	121.7 (d)	C-2, -6	H-2, H-5
5	7.01 (1H, d, *J*=8.0 Hz)	126.4 (d)	C-1, -3, -7	H-4
6		129.3 (s)		
7	3.17 (1H, m)	30.0 (d)	n.o.[e]	H_2-8, H_3-14
8/8´	1.86 (1H, m); 1.74 (1H, m)	36.8 (t)	C-6, -9	H-7, H_2-9
9/9´	1.69 (1H, m); 1.14 (1H, m)	25.7 (t)	C-10	H_2-8, H-10
10	2.85 (1H, dd, *J*=9.6, 2.4 Hz)	66.4 (d)	n.o.	H_2-9
11		59.0 (s)		
12	1.33 (3H, s)	24.8 (q)	C-10, -11, -13	
13	1.22 (3H, s)	18.4 (q)	C-10, -11, -12	
14	1.25 (3H, d, *J*=6.8 Hz)	22.5 (q)	C-6, -7, -8	H-7
15	2.26 (3H, s)	20.9 (q)	C-2, -3, -4	
OH-1	6.79 (3H, s)		C-1, -2, -6	

[a] Spectra recorded at 400 MHz in $CDCl_3$ at 25 °C. [b] Spectra recorded at 100 MHz in $CDCl_3$ at 25 °C. [c] *J* values (in Hz) in parentheses. [d] Multiplicity deduced by DEPT and indicated by usual symbols. [e] n.o.=not observed.

The gross structure of **1** was determined using 2D NMR studies. From the ^{1}H–^{1}H COSY spectrum of **1** (Figure 1 and Table 1), the separate coupling systems between H-2/H-4 (by allylic coupling); H-4/H-5; H-7/H_2-8/H_2-9/H-10; and H-7/H_3-14 were identified, which were assembled with the assistance of an HMBC experiment (Figure 1 and Table 1). The HMBC correlations between H-2/C-1, C-4, C-6; H-4/C-2, C-6; H-5/C-1, C-3, C-7; H_2-8/C-6, -9; H_2-9/C-10; H_3-12/C-10, C-11, C-13; and H_3-13/C-10, C-11, C-12, permitted elucidation of the carbon skeleton. A methyl attached at C-7 was established by the HMBC correlations between H_3-14 and C-6, C-7, C-8. The methyl proton signal at δ_H 2.26 (3H, s, H_3-15) was revealed by its HMBC correlations to C-2, C-3, and C-4, indicating its attachment to C-3. The presence of a hydroxy group attaching at C-1 was deduced from the HMBC correlations between a hydroxy proton (δ_H 6.79, 1H, s) with C-1, C-2, and C-6. Based on the above findings, the molecular framework of **1** was established unambiguously.

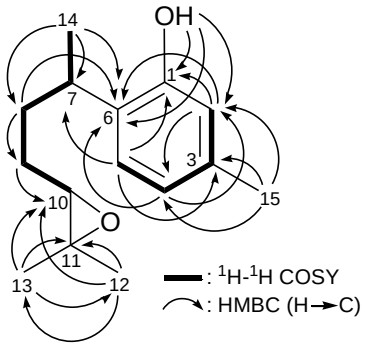

Figure 1. The ^{1}H–^{1}H COSY and HMBC Correlations of **1**

In a previous study, the structure of **1** as we presented in this paper had been prepared from (*S*)-(+)-curcuphenol and named as (7*S*,10*R*)-(+)-10,11-epoxycurcuphenol.[1] By detailed analysis, the spectral data of natural product **1** were found to be identical with those of the semi-synthetic product (7*S*,10*R*)-(+)-10, 11-epoxycurcuphenol. Since the absolute configurations for the C-7 and C-10 chiral carbons of this semi-synthetic product been determined as *S* and *R* forms, respectively,[1] we were able to assign the absolute configurations for the C-7 and C-10 chiral centers of natural product **1** as *S* and *R* form, respectively, by comparison the NMR data of C-7 methine (δ_H 3.17, 1H, m, H-7; δ_C 30.0, d, CH-7) and C-10 oxymethine (δ_H 2.85, 1H, dd, *J*=9.6, 2.4 Hz, H-10; δ_C 66.4, d, CH-10) for **1** with those of (7*S*,10*R*)-(+)-10,11-epoxycurcuphenol (δ_H 3.17, 1H, m, H-7; δ_C 30.2, d, CH-7; δ_H 2.85, 1H, dd, *J*=3.0, 9.3 Hz, H-10; δ_C 66.3, d, CH-10) and (7*S*,10*S*)-(+)-10,11-epoxycurcuphenol (δ_H 3.11, 1H, sext, *J*=6.9 Hz, H-7; δ_C 31.6, d, CH-7; δ_H 2.75, 1H, t, *J*=6.9 Hz, H-10; δ_C 64.6, d, CH-10).[1]

The known compound, (+)-curcuphenol (**2**), was first isolated from a Japanese marine sponge *Epipolasis* sp.,[2] and this compound was found to show interesting bioactivity.[2,3] The physical (rotation value) and

spectral data of **2** were in full agreement with those of reported previously.[2,3] To the best of our knowledge, sesquiterpenoid phenol **1** has not been isolated previously from any natural sources and (+)-curcuphenol (**2**) has not previously been reported from any octocoral.

In biological activity experiments, sesquiterpenoid phenols **1** and **2** displayed moderate and significant inhibitory effects on elastase release by human neutrophils, respectively, and these two metabolites also exhibited moderate inhibitory effects on superoxide anion release by human neutrophils (Table 2). In addition, compound **2** was found to exhibit moderate cytotoxicity toward the DLD-1 (human colon adenocarcinoma, ED_{50}=12.5 μg/mL) and CCRF-CEM (human T-cell acute lymphoblastic leukemia, ED_{50}=11.8 μg/mL) tumor cells, but **1** was inactive toward the above two cells (ED_{50}>40 μg/mL). All of these results suggest that small structural variations could influence the biological activities of the compounds of this type.

Table 2. Inhibitory Effects of Compounds **1** and **2** on Superoxide Anion Generation and Elastase Release by Human Neutrophils in Response to fMet-Leu-Phe/cytochalastin B

Compound	Superoxide Anion	Elastase
	IC_{50} (μM)[a] or (Inh.%)	IC_{50} (μM)[a] or (Inh.%)
1	(35.3±5.3)***	(38.8±6.8)**
2	(36.9±6.5)**	(83.6±5.3)***
DPI[b]	0.7±0.3	
PMSF[b]		131.4±12.7

Percentage of inhibition (Inh. %) at 10 μg/mL concentration. Results are presented as mean±S.E.M. (n=3–4). ** P<0.01, *** P<0.001 compared with the control value.
[a] Concentration necessary for 50% inhibition (IC_{50}).
[b] DPI (diphenylene indonium) and PMSF (phenylmethyl- sulfonyl fluoride) were used as positive control.

EXPERIMENTAL

General Experimental Procedures. Optical rotation values were measured with a JASCO P-1010 digital polarimeter. Infrared spectra were obtained on a VARIAN DIGLAB FTS 1000 FT-IR spectrometer. The NMR spectra were recorded on a VARIAN MERCURY PLUS 400 FT-NMR at 400 MHz for ^{1}H and 100 MHz for ^{13}C, in $CDCl_3$, respectively. Proton chemical shifts were referenced to the residual $CHCl_3$ signal (δ 7.26 ppm). ^{13}C NMR spectra were referenced to the center peak of $CDCl_3$ at δ 77.1 ppm. ESIMS and HRESIMS data were recorded on a BRUKER APEX II 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.

Animal Material. Specimens of the gorgonian coral *Echinomuricea* sp. were collected by hand using scuba gear off the southern Taiwan coast. This organism was identified by comparison with previous descriptions.[4] A voucher specimen has been deposited in the National Museum of Marine Biology & Aquarium (NMMBA), Taiwan.

Extraction and Isolation. The freeze-dried and minced material of *Echinomuricea* sp. (wet weight 1684 g, dry weight 428 g) was extracted with a mixture of MeOH and CH_2Cl_2 (1:1). The residue was partitioned between EtOAc and H_2O. The EtOAc layer was partitioned between MeOH and hexane. The hexane layer was separated by silica gel and eluted using hexane/EtOAc/MeOH to yield 21 fractions A–U. Fraction G was separated on silica gel and eluted using hexane/EtOAc (stepwise, 50:1–pure EtOAc) to yield 9 fractions, G1–G9. Fraction G4 was separated on silica gel and eluted with a mixture of hexane and CH_2Cl_2 to afford **2** (29.9 mg, hexane/CH_2Cl_2=4:1). Fraction G7 was further chromatographed on silica gel and eluted with a mixture of hexane and EtOAc to afford **1** (0.9 mg, hexane/EtOAc=11:1).

Sesquiterpenoid phenol 1 [(7*S*,10*R*)-(+)-10,11-Epoxycurcuphenol]: yellowish oil; $[\alpha]_D^{26}$ +46 (*c* 0.05, $CHCl_3$) (ref.[1]: $[\alpha]_D^{25}$ +15.5 (*c* 0.29, $CHCl_3$)); IR (neat) ν_{max} 3362 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) data, see Table 1; ESIMS *m/z* 257 $(M+Na)^+$; HRESIMS *m/z* 257.1516 (Calcd for $C_{15}H_{22}O_2$+Na, 257.1517). The spectral data of natural product **1** are in full agreement with those of reported previously.[1]

(+)-Curcuphenol (2): yellowish oil, the physical and spectral data of **2** are in full agreement with those of reported previously.[2,3]

Human Neutrophil Superoxide Anion Generation and Elastase Release. Human neutrophils were obtained by means of dextran sedimentation and Ficoll centrifugation. Superoxide generation and elastase release were carried out according to the procedure described previously.[5,6] 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.

Cytotoxicity Testing. The cytotoxicity of compounds **1** and **2** was assayed with a modification of MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric method. Cytotoxicity assays were carried out according to the procedures described previously.[7]

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