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3-Oxoabolene and 1-Oxocurcuphenol, Aromatic Bisabolanes from the Sponge *Myrmekioderma* sp.

Afsaneh Yegdaneh[a], Sumaitt Putchakarn[b], Supreeya Yuenyongsawad[c], Alireza Ghannadi[a] and Anuchit Plubrukarn[c,*]

[a]*School of Pharmacy*, *Isfahan University of Medical Sciences*, *Isfahan 81745*, *Iran*

[b]*Institute of Marine Science*, *Burapha University*, *Chonburi 20131*, *Thailand*

[c]*Department of Pharmacognosy and Pharmaceutical Botany*, *Faculty of Pharmaceutical Sciences*, *Prince of Songkla University*, *Hat-Yai*, *Songkhla 90112*, *Thailand*

anuchit.pl@psu.ac.th

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Two new aromatic bisabolane sesquiterpenes possessing an oxo functionality on the prenyl chain, (+)-3-oxoabolene (**3**) and (+)-1-oxocurcuphenol (**4**), along with two known sesquiterpenes, (+)-curcuphenol (**1**) and (+)-curcudiol (**2**), were isolated from the sponge *Myrmekioderma* sp. The antiproliferative activity of **2**-**4** was determined and showed an interesting selectivity; i.e., a good activity against HT-29 cells with IC_{50}s in the μM range, but a weak and incalculable toxicity against Hela and normal fibroblast cells.

Keywords: *Myrmekioderma*, Bisabolane, Curcuphenol, Antiproliferation.

Curcuphenol (**1**) and related bisabolane sesquiterpenes are among a few groups of biologically active metabolites that have been identified both from terrestrial plants and marine invertebrates. In plants, **1** is found as one component of volatile oils from several aromatic herbs, including *Curcuma aromatica*, *C. angustifolia*, *Callicarpa japonica*, and *Lindera pulcherrima* [1]. On the other hand, **1** and related bisabolanes have been isolated from sponges of the genera *Epipolasis* [2], *Myrmekioderma* [3], *Didiscus*, [4], and *Arenochalina* [5], and from gorgonians of the genera *Pseudopterogorgia* [6] and *Echinomuricea* [7]. A specimen of the sponge *Myrmekioderma* sp. collected from Phi Phi Islands National Park, Krabi Province, was screened for biological activity and showed cytotoxicity against a panel of cancer cell lines. Chemical investigation of the sponge led to the isolation of two known compounds, **1** and curcudiol (**2**) [2,4a,6a], along with two new aromatic bisabolanes, 3-oxoabolene (**3**) and 1-oxocurcuphenol (**4**) (Figure 1).

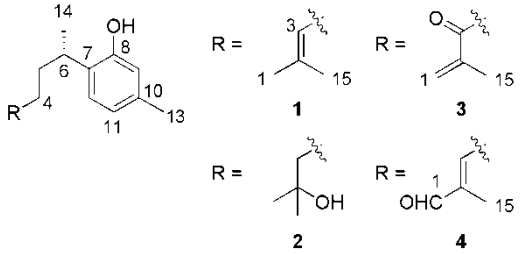

Figure 1: Chemical structures of compounds **1**-**4**.

Table 1: NMR spectral data of **3** and **4** ($CDCl_3$; 500 MHz for ^1H, 125 MHz for ^{13}C).

position	**3**		**4**	
	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
1a	5.99 (1H, s)	125.7, CH_2	9.35 (1H, s)	195.4, CH
b	5.79 (1H, br s)			
2		144.3, C		138.8, C
3		203.8, C	6.44 (1H, tq, 7.3, 1.4)	155.1, CH
4a	2.85 (1H, ddd, 17.6, 9.1, 4.6)	34.5, CH_2	2.26 (1H, m)	35.5, CH_2
b	2.66 (1H, ddd, 17.6, 6.4, 4.8)		1.38 (1H, m)	
5a	1.93 (1H, dddd, 14.4, 9.2, 5.0, 4.8)	32.4, CH_2	1.82 (1H, m)	29.6, CH_2
b	1.53 (1H, m)		1.73 (1H, m)	
6	2.99 (1H, tq, 6.9, 6.6)	30.5, CH	3.08 (1H, tq, 6.4, 6.8)	31.8, CH
7		128.9, C		128.1, C
8		154.1, C		152.8, C
9	6.71 (1H, br s)	116.9, CH	6.53 (1H, d, 0.9)	116.1, CH
10		137.1, C		137.1, C
11	6.68 (1H, d, 7.7)	120.9, CH	6.71 (1H, dd, 7.3, 0.9)	121.8, CH
12	7.01 (1H, d, 7.7)	125.7, CH	7.01 (1H, d, 7.3)	127.1, CH
13	2.27 (3H, s)	20.9, CH_3	2.25 (3H, s)	20.8, CH_3
14	1.24 (3H, d, 6.6)	18.9, CH_3	1.23 (3H, d, 6.8)	21.0, CH_3
15	1.88 (3H, s)	17.6, CH_3	1.65 (3H, br s)	9.1, CH_3
8-OH	7.11 (1H, br s)			

The molecular formula of **3** was proposed to be $C_{15}H_{20}O_2$ from the HR-ESI mass spectral analysis. This requires an unsaturation degree of 6, belonging to an aromatic ring, a keto and an olefin, as indicated by the ^1H and ^{13}C NMR spectra (Table 1). The aromatic moiety is recognized to be a 1,2,4-trisubstituted phenyl group, characterized by three protons at δ 7.01 (d, *J* = 7.7 Hz, H-12), 6.71 (br s, H-9) and 6.68 (d, *J* = 7.7 Hz, H-11). HMBC correlations (Figure 2) from H-9 and H-11 to C-13 (δ 20.9), and from H-13 (δ 2.27, s) to C-9 (δ 116.9), C-10 (δ 137.1), and C-11 (δ 120.9), allowed the establishment of a methyl group substituted on C-10. In the same manner, the substitution of an alkyl group on C-7 and a hydroxy on C-8 were simultaneously indicated by the HMBC correlations from H-12 to C-6 (δ 30.5) and C-8 (δ 154.1), and from H-11 and H-14 (δ 1.24, d, *J* = 6.6 Hz) to C-7 (δ 128.9). The presence of the hydroxy group was also confirmed by the exchangeable OH resonance at δ 7.11 (br s). On the other end, a vinyl keto terminal composed of keto (δ_C 203.8, C-3), and vinyl groups (δ_C 144.3, C-2, and 125.7, C-1; δ_H 5.99, s, H-1a; 5.79, br s, H-1b) was elucidated. HMBC correlations from H-15 (δ 1.88, s) to C-1, C-2, and C-3 allowed the placement of a methyl group on C-2. This keto vinyl moiety connects to the aforementioned phenyl ring via a propylene bridge (δ_C 34.5, C-4; 32.4, C-5; 30.5, C-6; δ_H 2.99, tq, *J* = 6.9, 6.6 Hz, H-6; 2.85, ddd, *J* = 17.6, 9.4, 4.6 Hz, H-4a; 2.66, ddd, *J* = 17.6, 6.4, 4.8 Hz, H-4b; 1.93, dddd, *J* = 14.4, 9.2, 5.0, 4.8 Hz, H-5a; 1.53, m, H-5b), as indicated also by HMBC correlations

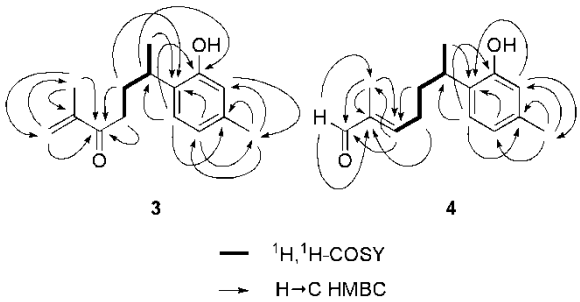

Figure 2: ^{1}H,^{1}H-COSY and key HMBC (H→C) correlations of **3** and **4**

from H-4 and H-5 to C-3, and from H-6 to C-7 and C-8. The conventional quartet of H-6 also indicated the substitution of a methyl group (δ_H 1.24, d, J = 6.6 Hz, H-14). The structure of **3** was therefore proposed to be a new bisabolane sesquiterpene, 3-oxoabolene.

Compound **4** also has a molecular formula of $C_{15}H_{20}O_2$, as indicated by the HR-ESI mass spectrum. The moiety of a 1,2,4-trisubstituted phenyl ring composed of H-12 (δ 7.01, d, J = 7.5 Hz), H-11 (δ 6.71, dd, J = 7.3, 0.9 Hz), and H-9 (δ 6.53, d, J = 0.9 Hz), similar to that of **3**, was recognized in the NMR spectrum (Table 1). Substitution of a methyl (C-13, δ 20.9), a hydroxyl, and all four adjacent carbons of the alkyl side chain (C-4 - C-6, and C-14) was elucidated by means of HMBC correlation analysis (Figure 2) in the same manner to that for **3**. Through the ^{1}H,^{1}H coupling between H-3 and H-4 (Figure 2), the prenyl terminal of **4** ends with an α,β-unsaturated aldehyde (δ_C 195.4, C-1; 155.1, C-3; 138.8, C-2; δ_H 9.35, s, H-1; 6.44, tq, J = 7.3, 1.4 Hz, H-3). HMBC correlations from H-15 (δ 1.65, br s) to C-1, C-2, and C-3 also connected this methyl group on C-2. Compound **4** is proposed to be a new aromatic bisabolane, 1-oxocurcuphenol.

With the limited amount of **4**, the NOE experiments to determine the geometry of the terminal olefin did not yield analyzable results. The geometry of Δ^2 olefin was proposed here to be an *E* on the basis of the up-field chemical shift of C-15 at 9.1 ppm. It has been observed that, for a trisubtituted *E* olefin on which a methyl and a carbonyl reside on the α carbon, the alkyl substation on the β end would cause repulsion in such a way that the methyl carbon generally resonates at a high-fielded region of 10-13 ppm. On the other hand, with the less repulsed *Z* counterpart, the α-methyl resonates at the lower field of 20 ppm [8].

The specific rotations of the samples of **1** and **2** isolated in this investigation (+14.3, *c* 2.5, CH_3OH; and +1.7, *c* 0.205, CH_3OH, respectively) were dextrorotatory with comparable magnitudes to the previous reported data [2], and indicated a 7-*S* configuration for both. Based on the assumption that they came from the same biological source, the configuration of **3** and **4** was proposed to be 7-*S*. This agrees well with the observation that marine-derived curcuphenols, sponges [2-4] and the *Echinomuricea* gorgonian [7] are the sources of 7-*S* enantiomers, whereas the 7-*R* has been found in *Pseudopterogorgia* gorgonians [6] and in certain plants, for example, *Lasianthaea podocephala* [9]. It is also worth mentioned that aromatic bisabolanes bearing an oxo functionality at any position on the prenyl chain are surprisingly rare. To our knowledge, there has been only one report of a natural 4-oxo analog from a *Myrmekioderma* sponge [3b], whereas a few other oxo derivatives are microbial-transformed products using **1** as a precursor [10].

Compounds **2**, **3**, and **4** were active against HT-29 cancer cells (IC_{50}s 0.011±0.001, 0.67±0.17, and 0.017±0.005 μM, respectively). The activity against HeLa and human normal fibroblasts of the three compounds was very weak (lower than 50% proliferative inhibition at the highest concentration of 1 mg/mL). Unfortunately, compound **1** decomposed accidentally prior to being subjected to the activity determination, and our attempt to recover the compound was unsuccessful. The selectivity of the bisabolanes against specific cell lines has already been observed [4a,11]. With the relatively low toxicity against normal cells, such selectivity may open up the possibility to modify the aromatic bisabolane derivatives towards better selective antiproliferative agents.

Experimental

General: Optical rotations, Perkin Elmer 341 polarimeter; UV, Genesys10 spectrophotometer; IR, Jasco810 spectrophotometer; 1D and 2D NMR, Varian Unity INOVA500 spectrometer; ESI-MS, both low- and high-resolution, LCT Waters Micromass 2690 mass spectrometer. HPLC, Thermo-Finnigan Spectra system controller SCM 1000, P4000 quaternary pump, UV6000LP diode array detector, and Rheodyne 7725i injector port, operated with ChromQuest 4.2.34 (3.1.6). Silica gel 60 (Scharlau) was used for CC, and precoated silica gel 60 F_{254} (Merck) visualized with anisaldehyde/H_2SO_4 for TLC.

Animal material: The sponge specimen of *Myrmekioderma* sp. (family Heteroxyidae) was collected in the vicinity of Phi Phi Islands National Park, Krabi Province. The specimen was kept in an ice chest (0°C) upon surfacing and at -20°C upon arrival at the lab until the isolation. The taxonomic identification was performed by us (S.P.), and the voucher specimens were lodged at the Institute of Marine Science, Burapha University (BIMS-I2004), and at the Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Prince of Songkla University (PHID-A01).

Extraction and isolation: The sponge (700 g wet weight) was macerated in a 1:1 CH_3OH/EtOAc mixture (4×500 mL). The extract, after evaporation and partitioning with EtOAc to remove traces of water, was fractionated successively to yield *n*-hexane-, CH_2Cl_2- and *n*-BuOH-extracts. The *n*-hexane-extract was chromatographed over a silica gel column (*n*-hexane/EtOAc/CH_2Cl_2 8.5:0.5:1), then with C18 RP-HPLC (Phenomenex, 10 μm, 10×250 mm, 25% H_2O in CH_3CN) to yield **1** (101 mg). The CH_2Cl_2-extract was fractionated over a silica gel column (*n*-hexane/EtOAc/CH_2Cl_2 1:2:8). After appropriate fractions were pooled, two major fractions were selected for further isolations. The first one was fractionated over a silica gel column (1% CH_3OH in CH_2Cl_2) to yield **2** (20 mg). Another main fraction was chromatographed over a C18 RP-HPLC column (Phenomenex, 10 μm, 10×250 mm, 40% H_2O in CH_3CN), and **3** (2 mg) and **4** (1.5 mg) was obtained.

(+)-3-Oxoabolene (**3**)
White amorphous solid
$[\alpha]_D$: +1.5 (*c* 0.125, CH_3OH).
IR (KBr): 3500-3300 (br), 1660, 1620, 1460, 1420 cm^{-1}.
UV (CH_3OH) λ_{max} (log ε): 218 (8.64), 278 (7.92) nm.
^{1}H and ^{13}C NMR: Table 1.
MS (ESI): *m/z* (%) 487 [2M + Na^+] (12), 255 [M + Na^+] (100).
HRMS-ESI: *m/z* [2M + Na^+] calcd for $C_{30}H_{40}O_4Na$: 487.2814; found: 487.2808.

(+)-1-Oxocurcuphenol (**1**)
White amorphous solid

$[\alpha]_D$: +6.7 (*c* 0.075, CH_3OH).
IR (KBr): 3500-3300 (br), 1729, 1685, 1612, 1456 cm^{-1}.
UV (CH_3OH) λ_{max} (log ε): 214 (7.84), 278 (7.01) nm.
^1H and ^{13}C NMR: Table 1.
HRMS-ESI: *m/z* [M + H^+] calcd for $C_{15}H_{21}O_2$: 233.1536; found: 233.1523.

Antiproliferative activity: The antiproliferative activity was determined using the sulphorhodamine B colorimetric assay [12], targeting HT-29 human colon adenocarcinoma, HeLa human cervical carcinoma, and human normal gingival fibroblast cells. The activity was expressed as an IC_{50}±SD, determined from regression of the linear portions of the dose-response plots, and referenced camptothecin as a standard drug (IC_{50}s against HT-29 and HeLa cells 0.008±0.015 and 0.28±0.06 μM, respectively.)

Supplementary data: Underwater photograph and description for microscopic characteristics of the sponge specimens, and 1D ^1H and ^{13}C NMR, HMQC, and HMBC spectra of **3** and **4** are available.

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References

[1] (a) Kojima H, Yanai T, Toyota A. (**1998**) Essential oil constituents from Japanese and Indian *Curcuma aromatica* rhizomes. *Planta Medica*, ***64***, 380-381; (b) Kobaisy M, Tellez MR, Dayan FE, Duke SO. (**2002**) Phytotoxicity and volatile constituents from leaves of *Callicarpa japonica* Thumb. *Phytochemistry*, ***61***, 37-40; (c) Srivastava AK, Srivastava SK, Syamsundar KV. (**2006**) Volatile composition of *Curcuma angustifolia* Roxb. rhizome from central and southern India. *Flavour and Fragrance Journal*, ***21***, 423-426; (d) Joshi SC, Padalia RC, Bisht DS, Mathela CS. (**2009**) Terpenoid diversity in the leaf essential oils of Himalayan Lauraceae species. *Chemistry & Biodiversity*, ***6***, 1364-1673.

[2] Fusetani N, Suguno M, Matsunaga S, Hashimoto. (**1987**) (+)-Curcuphenol and dehydrocurcuphenol, novel sesquiterpenes which inhibit H,K-ATPase, from a marine sponge *Epipolasis* sp. *Experientia*, ***43***, 1234-1235.

[3] [a] Peng J, Franzblau SG, Zhang F, Hamann MT. (**2002**) Novel sesquiterpenes and a lactone from the Jamaican sponge *Myrmekioderma styx*. *Tetrahedron Letters*, ***43***, 969-9702; (b) Letourneux Y, Brunel JM, Fernandez R, Dherbomez M, Debitus C. (**2005**) Isolation and characterization of new tetrahydropyranyl substituted sesquiterpene and myrmekiodermin glycolipid ether isolated from the marine sponge *Myrmekioderma*. *Heterocyclic Communications*, ***11***, 291-298.

[4] (a) Wright AE, Pomponi SA, McConnell OJ, Kohmoto S, McCarthy PJ. (**1987**) (+)-Curcuphenol and (+)-curcudiol, sesquiterpene phenols from shallow and deep water collections of the marine sponge *Didiscus flavus*. *Journal of Natural Products*, ***50***, 976-978; (b) Gasper H, Feio SS, Rodrigues AI, Van Soest R. (**2004**) Antifungal activity of (+)-curcuphenol, a metabolite from the marine sponge *Didiscus oxeata*. *Marine Drugs*, ***2***, 8-13.

[5] Butler MS, Capon RJ, Nadeson R, Beveridge AA. (**1991**) Aromatic bisabolenes from an Australian marine sponge, *Arenochalina* sp. *Journal of Natural Products*, ***54***, 619-623.

[6] (a) McEnroe FJ, Fenical W. (**1978**) Structures and synthesis of some new antibacterial sesquiterpenoids from the gorgonian coral *Pseudopterogorgia rigida*. *Tetrahedron*, ***34***, 1661-1664; (b) Miller SL, Tinto WF, McLean S, Reynolds WF, Yu M. (**1995**) Bisabolane sesquiterpenes from Barbadian *Pseudopterogorgia* spp. *Journal of Natural Products*, ***58***, 1116-1119; (c) Freyer AJ, Patil AD, Killmer L, Zuber G, Myers C, Johnson RK. (**1997**) Rigidone, a sesquiterpene *o*-quinone from the gorgonian *Pseudopterogorgia rigida*. *Journal of Natural Products*, ***60***, 309-311; (d) D'Armus HT, Mootoo BS, Reynolds WF. (**2000**) An unusual sesquiterpene derivatives from the Caribbean gorgonian *Pseudopterogorgia rigida*. *Journal of Natural Products*, ***63***, 1593-1595.

[7] Chung H-M, Hwang T-L, Wang W-H, Fang L-S, Sung P-J. (**2009**) Curcuphenol derivatives from the gorgonian *Echinomuricea* sp. *Heterocycles*, ***78***, 2595-2600.

[8] (a) Yang Y-H, Fu X-L, Li L-Q, Zeng Y, Li C-Y, He L-N, Zhao P-J. (**2012**) Naphthomycins L-N, ansamycin antibiotics from *Streptomyces* sp. CS. *Journal of Natural Products*, ***75***, 1409-1413; (b) Pan L, Acuna UM, Li J, Jena N, Ninh TN, Pannell CM, Chai H, Fuchs JR, Carcache de Blanco EJ, Soejarto DD, Kinghorn AD. (**2013**) Bioactive flavaglines and other constituents isolated from *Aglaia perviridis*. *Journal of Natural Products*, ***76***, 394-404; (c) Wang K-W, Wang S-W, Du Q-Z. (**2013**) Complete NMR assignment of retinal and its related compounds. *Magnetic Resonance in Chemistry*, ***51***, 435-438.

[9] (a) Ghisalberti EL, Jefferies PR, Stuart AD. (**1979**) The absolute configuration of a sesquiterpene enone from *Stevia purpurea* and a phenol from *Lasianthaea podocephala*. *Australian Journal of Chemistry*, ***32***, 1627-1630; (b) Gul W, Hammond NL, Yousef M, Peng J, Holley A, Hamann MT. (**2007**) Chemical transformation and biological studies of marine sesquiterpene (*S*)-(+)-curcuphenol and its analogs. *Biochimica et Biophysica Acta*, ***1770***, 1513-1519.

[10] El Sayed KA, Yousef M, Hamann MT, Avery MA, Kelly M, Wipf P. (**2002**) Microbial and chemical transformation studies of the bioactive marine sesquiterpenes (*S*)-(+)-curcuphenol and –curcudiol isolated from a deep reef collection of the Jamaican sponge *Didiscus oxeata*. *Journal of Natural Products*, ***65***, 1547-1553;

[11] Rodrigo G, Almanza GR, Cheng Y, Peng J, Hamann M, Duan R-D, Åkesson B. (**2010**) Antiproliferative effects of curcuphenol, a sesquiterpene phenol. *Fitoterapia*, ***81***, 762-766.

[12] Skehan P, Storeng R, Scudiero D, Monks A, McMahon J, Vistica D, Warren JT, Bokesch H, Kenney S, Boyd MR. (**1990**) New colorimetric cytotoxicity assay for anticancer-drug screening. *Journal of the National Cancer Institute*, ***82***, 1107-1112.