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TETRAHEDRON

New Hirsutane Based Sesquiterpenes from Salt Water Cultures of a Marine Sponge-Derived Fungus and the Terrestrial Fungus *Coriolus consors*

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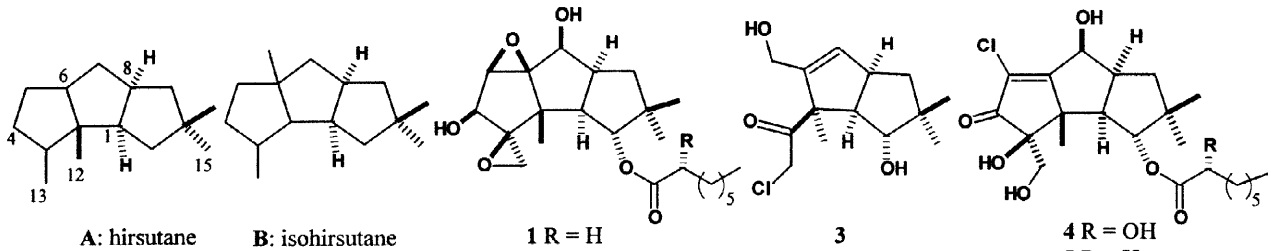
Abstract: Five new cyclic sesquiterpenes, hirsutanols A (**6**), B (**7**), C (**8**), hirsutanol D (**9**), and *ent*-gloeosteretriol (**10**) and a new diketopiperazine (**14**) were isolated from salt water cultures of two fungi. Sesquiterpenes **6** - **8** and **10** were obtained from an unidentified fungus separated from an Indo-Pacific sponge *Haliclona* sp. while **9** and **14** were produced by the terrestrial fungus *Coriolus consors* cultured under both sea water and deionized water media. Hirsutanol A (**6**) and *ent*-gloeosteretriol (**10**) were found to be anti-microbial (*Bacillus subtilus*) active. All structures were elucidated by spectroscopic methods.

INTRODUCTION

In 1994, we reported the isolation and characterization of hirsutane (**A**)[1] framework sesquiterpenes from the salt water fermentation of an unidentified sponge-derived fungus.[2] The unique aspect of this work was that the cultures were obtained from the common Indo-Pacific sponge *Jaspis* cf. *johnstoni*, a regular source of jasplakinolide.[3] Scale-up salt water culture of this fungus afforded broth mixtures containing hirsutane-type sesquiterpenes but no ketide-amino acids (such as jasplakinolide) could be detected. The sesquiterpenes isolated were coriolin B (**1**) and dihydrocoriolin C (**2**), both originally isolated and studied biosynthetically from the terrestrial fungus *Coriolus consors*.[4] Also obtained from the mycelium were three new Cl-containing compounds named chloriolins A - C (**3** - **5**).[2] While continuing this avenue of research we have found that chemically prolific fungi can be regularly obtained from marine sponges. Currently, our belief is that sponges, which have provided about 41% of all bioactive marine derived compounds,[5] ought to be an important source



A: hirsutane **B**: isohirsutane **1** R = H, **2** R = OH **3** **4** R = OH, **5** R = H

of chemically prolific fungi.[6,7] Furthermore, there appears to be continuing interest in the overall chemistry of fungi because this group is among the world's greatest untapped resources for new biodiversity as well as chemodiversity.[8]

The isolation of coriolins and chloriolins from a marine sponge-derived fungus was the stimulus for additional experiments along several lines. We wanted to explore the possibility that other coriolin producing

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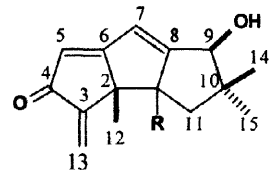
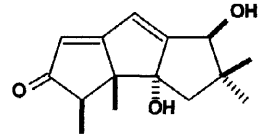
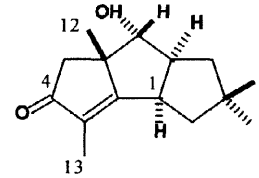
fungi could be obtained from sponges. The prospects that ATCC-derived specimens of *Coriolus consors*[9] could incorporate Cl into hirsutane biosynthetic products when grown in salt water was also of interest. We now report progress relevant to these issues. In particular, a *Haliclona* species of sponge has yielded an unidentified fungus which under salt water fermentation conditions yields new hirsutane (**A**) sesquiterpenes: **6** - **8** and **10**.[10] The salt water fermentation of an ATCC-derived culture of *Coriolus consors* provided a compound **9**[10] having the new isohirsutane (**B**) skeleton accompanied by diketopiperazines **13** and **14**. Unfortunately, no chlorinated compounds could be observed from the fungi we explored that proved to be rich in hirsutane or isohirsutanes.



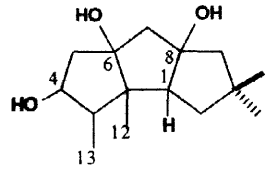
hirsutanol A (**6**) R=α-OH
hirsutanol B (**7**) R=β-OH



hirsutanol C (**8**)



hirsutanol D (**9**)



ent-gloeosteretriol (**10**)
H1=β, OH4=α, OH6=α, OH8=β, Me12=α, Me13=β
gloeosteretriol (**11**)
H1=α, OH4=β, OH6=β, OH8=α, Me12=β, Me13=α
anthrosporol (**12**)
H1=α, OH4=α, OH6=β, OH8=α, Me12=β, Me13=β

RESULTS AND DISCUSSION

During an expedition to Indonesia (Tomini Bay, North Sulawesi (N00°15.831′, E122°05.690′)) we cultured a fungus sample 95-1005C separated from *Haliclona* sp. sponge. A 125 mL culture broth provided an extract showing selectivity *in vitro* against solid tumor cells.[11] Thus, the ethyl acetate extract of an 8 L broth was solvent partitioned and subsequent chromatographic fractions were purified to yield hirsutanol A (**6**), B (**7**), C (**8**) and *ent*-gloeosteretriol (**10**). Hirsutanol D (**9**) and diketopiperazines **13** and **14** were isolated by a similar procedure (including normal phase HPLC) from a 0.5 L salt water culture broth of various ATCC-derived strains of *Coriolus consors*.[9]

^{1}H NMR data for coriolins and chloriolins provide an important reference point for recognizing compounds containing the A framework. The diagnostic ^{1}H NMR fingerprint includes two geminal methyls at δ 0.9 - 1.1 and δ 1.1 - 1.3 and another methyl at δ 0.8 - 1.3 all as singlet signals. Additionally, geminal proton resonances occur between δ 1.6 - 1.8 and often other geminal proton resonances are observed downfield from δ 2.0 with a large coupling (e.g. J = 14.5 Hz). This information was applied to rapidly establish structures **6** - **8** and **10**.

The APT ^{13}C NMR spectrum of **6** suggested that three methyls, two methylenes, three methines and seven quaternary carbons were present in the molecule. An APT formula of $C_{15}H_{16}$ agreed with the HRFABMS molecular formula of $C_{15}H_{18}O_3$ based on m/z = 247.1335 [$(M+H)^+$, Δ -0.1 mmu of calc]. The ^{13}C NMR spectrum, tabulated in Table 1, showed seven downfield carbon signals at δ 197.5 (s), 189.9 (s), 173.4 (s), 148.8 (s), 119.8 (d), 116.8 (d), and 113.5 (t). HMBC correlations (H5 to C3, C4, C6, C7 and C2; H7 to C1, C2 and C6; H9 to C7 and C8; and H13 to C2, C3 and C4) defined a partial structure C13-C3-C4-C5-C6-C7-C8-C9, which included a cross-conjugated trienone moiety. Also consistent with this array were ^{1}H NMR signals listed in Table 2: terminal methylene δ 5.27 (d, J = 1.0 Hz) and 6.00 (d, J = 1.0 Hz); and vinyl protons

δ 6.02 (s) and 6.50 (d, J = 2.5 Hz). The remaining degrees of unsaturation were accounted for by proposing three fused rings of the hirsutane (**A**) framework. The C9 - C10 - (Me14, Me15)-C11-C1-C2 - (Me12) fragment within **A** was deduced from HMBC correlations (H9 to C10, C14, C15, and H11/H11′ to C1, C2, C8, C9, and C10. Subtracting a carbonyl (δ 197.5, s) oxygen from the molecular formula still required two alcohols which, based on ^{13}C shifts, were attached to C9 (δ 76.5) and C1 (δ 83.2). The final assignment of all protonated carbons, verified by HMQC data, was consistent with the 2D structure as shown for **6**.

With the gross structure of hirsutanol A (**6**) established, attention was then turned to defining its relative stereochemical relationships. A NOESY experiment provided diagnostic correlations, shown in Figure 1, including from Me15 to H11α and to H9, and from H11β to Me12 and to Me14β, which indicated that these sets of protons were on opposite molecular faces, respectively. Also, nOe correlations seen in dioxane-d_8 solution from OH1 to H9 and H11α meant that these three groups were on the same molecular face. Finally, hirsutanol A (**6**) was found to be active against *Bacillus subtilus*.[12]

Table 1. ^{13}C NMR chemical shifts for hirsutanol A (**6**), B (**7**), C (**8**), D (**9**) and *ent*-gloeosteretriol (**10**) (125 MHz).

C	6[a]	7[a]	8[a]	9[b]	10[a]
1	83.2 (s)	87.7 (s)	84.1 (s)	40.5 (d)	56.8 (d)
2	61.8 (s)	61.6 (s)	63.5 (s)	182.0 (s)	55.2 (s)
3	148.8 (s)	148.4 (s)	48.0 (d)	131.3 (s)	52.5 (d)
4	197.5 (s)	197.2 (s)	214.4 (s)	208.0 (s)	76.9 (d)
5	119.8 (d)	121.0 (d)	118.4 (d)	50.9 (t)	49.9 (t)
6	189.9 (s)	189.9 (s)	195.0 (s)	49.8 (s)	89.8 (s)
7	116.8 (d)	120.3 (d)	118.0 (d)	76.2 (d)	54.5 (t)
8	173.4 (s)	170.7 (s)	174.6 (s)	47.9 (d)	87.7 (s)
9	76.5 (d)	76.1 (d)	77.9 (d)	39.4 (t)	57.9 (t)
10	42.6 (s)	45.5 (s)	44.0 (s)	43.0 (s)	42.1 (s)
11	44.1 (t)	43.8 (t)	45.3 (t)	43.6 (t)	44.9 (t)
12	26.6 (q)	26.0 (q)	24.3 (q)	20.4 (q)	18.6 (q)
13	113.5 (t)	113.9 (t)	9.6 (q)	6.1 (q)	12.5 (q)
14	23.0 (q)	21.3 (q)	23.8 (q)	26.5 (q)	30.6 (q)
15	28.8 (q)	28.4 (q)	30.2 (q)	24.8 (q)	28.6 (q)

[a] CD_3OD; [b] $CDCl_3$

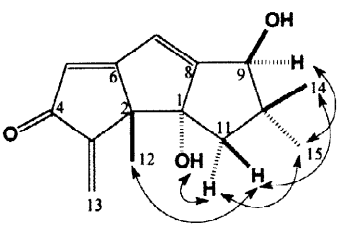


Figure 1. Important NOESY correlations for hirsutanol A (**6**) in dioxane-d_8

Once hirsutanol B (**7**) was isolated, we quickly recognized from the ^1H and ^{13}C NMR data that it was an epimer of hirsutanol A (**6**). Small differences were noted in the ^1H NMR δs between **7** and **6** (see Table 2) including a 0.41 ppm shift variation for H9 and 0.61 and 0.10 ppm, for H11/H11′, respectively. On the other hand, significant differences were evident in the ^{13}C NMR shifts of C1 for **7** (δ 87.7) and **6** (δ 83.2) which strongly suggested that C1 was the epimeric carbon. Unfortunately, this compound decomposed before nOe measurements could be undertaken.

Another member of this series, hirsutanol C (**8**) was characterized next. The HRFABMS indicated its molecular formula of $C_{15}H_{20}O_3$ [m/z = 249.1494 $(M+H)^+$, Δ -0.3 mmu of calc]. The ^1H and ^{13}C NMR spectral profiles for hirsutanol C (Tables 1 and 2) suggested it was a new member of the hirsutanol family. The ^1H NMR of **8**, in comparison to hirsutanol A (**6**), included additional resonances: a methyl at δ 1.06 (d, J = 7.0 Hz), a methine at δ 2.90 (q, J = 7.0 Hz). These data along with the absence of terminal double bond signals led to the proposal of structure **8** for hirsutanol C. ^{13}C NMR δs and data from an nOe experiment indicated the

stereochemistry at C1, C2, and C9 was the same as that in hirsutanol A (**6**). Furthermore, nOe correlations were also observed between H11β and $CH_3$12 and $CH_3$13.

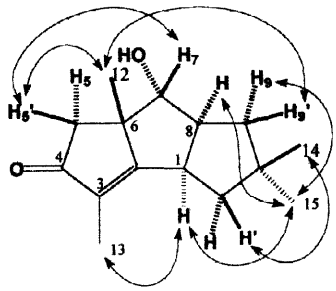


Figure 2. NOESY correlations for hirsutanol D (**9**) in $CDCl_3$.

The next compound analyzed, hirsutanol D (**9**), obtained from *C. consors* (ATCC #66132), had a rather different orientation of the four methyls groups about the triquinane ring. The molecular formula of $C_{15}H_{22}O_2$ was established from HRFABMS data [*m/z* = 235.1698 $(M+H)^+$, Δ 0.0 mmu of calc]. Diagnostic 1D NMR resonances suggested the presence of a fused triquinane ring system but did not allow immediate distinction between systems **A** and **B**. The resonances of mutually coupled spins H11/11′-H1-H8-H7-H9/H9′ in the ^1H NMR spectrum (Table 2, and confirmed by a COSY experiment) allowed assignment of the eastern two rings of **9**. A carbonyl group (δ 208) with α,β unsaturation (δ 131, 182) was also evident. The HMBC correlations from $H_3$12 to C2, C5, C7; $H_3$13 to C2, C3, C4; H11′ to C14; and $H_3$14 to C15 provided information to attach all methyl groups and determine the planar structure of **9**. The OH placed at C7 (δ 76.2) was justified by coupling to H7 (δ 4.0) observed in a ^1H NMR spectrum obtained in dioxane-d_8. Finally, the stereochemistry shown for **9** was deduced using NOESY correlations shown in Figure 2.

Table 2. ^1H NMR chemical shifts for hirsutanol A (**6**), B (**7**), C (**8**), D (**9**) and *ent*-gloeosteretriol (**10**) (500 MHz).

H	6[a]	7[a]	8[a]	9[b]	10[a]
1				3.36 (q, *J*=10.0 Hz)	2.31 (t, *J*=9.5 Hz)
3			2.90 (q, *J*=7.0 Hz)		1.62 (1H, m)
4					3.95 (q, *J*=8.5 Hz)
5	6.02 (1H, s)	6.06 (1H, s)	5.76 (1H, s)	2.35 (d, *J*=17.0 Hz)	2.63 (dd, *J*=8.5, 14.0 Hz)
				2.29 (d, *J*=17.0 Hz)	1.61 (dd, *J*=8.5, 14.0 Hz)
7	6.50 (d, *J*=2.5 Hz)	6.64 (d, *J*=1.0 Hz)	6.44 (d, *J*=2.5 Hz)	4.00 (d, *J*=9.0 Hz)	2.03 (d, *J*=13.5 Hz)
					1.82 (d, *J*=13.5 Hz)
8				3.15 (p, *J*=9.0 Hz)	
9	4.63 (d, *J*=2.5 Hz)	4.22 (d, *J*=1.0 Hz)	4.65 (d, *J*=2.5 Hz)	1.90 (dd, *J*=12.5, 11.0 Hz)	1.67 (d, *J*=13.5 Hz)
				1.56 (ddd, *J*=13.5, 9.0, 2.5 Hz)	1.50 (d, *J*=13.5 Hz)
11	2.67 (d, *J*=14.5 Hz)	2.06 (d, *J*=14.5 Hz)	2.10 (d, *J*=15.0 Hz)	1.88 (dd, *J*=12.5, 9.0, 2.5 Hz)	1.42 (2H, m)
	1.71 (d, *J*=14.5 Hz)	1.81 (d, *J*=14.5 Hz)	1.61 (d, *J*=15.0 Hz)	1.61 (dd, *J*=12.5, 10.0 Hz)	
12	1.24 (3H, s)	1.18 (3H, s)	1.03 (3H, s)	1.31 (3H, s)	0.86 (3H, s)
13	6.00 (d, *J*=1.0 Hz)	5.99 (d, *J*=0.5 Hz)	1.06 (d, *J*=7.0 Hz)	1.69 (3H, s)	0.96 (d, *J*=7.0 Hz)
	5.27 (d, *J*=1.0 Hz)	5.29 (d, *J*=0.5 Hz)			
14	0.95 (3H, s)	1.07 (3H, s)	0.93 (3H, s)	1.18 (3H, s)	1.01 (3H, s)
15	1.29 (3H, s)	1.27 (3H, s)	1.29 (3H, s)	1.05 (3H, s)	1.11 (3H, s)

[a] CD_3OD, [b] $CDCl_3$

The final hirsutane type compound, *ent*-gloeosteretriol (**10**), was isolated from a sponge-derived fungus fermentation broth. Its molecular formula was established as $C_{15}H_{26}O_3$ based on mass spectra [EIMS *m/z* = 254.2 $(M)^+$, HRCIMS *m/z* = 237.1855 $(M-H_2O+H)^+$, Δ 0.0 mmu of calc] and NMR data (Tables 1 and 2). The three degrees of unsaturation of **10** were satisfied by the three fused five-membered rings. The APT ^{13}C NMR data suggested the presence of four methyls, four methylenes, three methines and four quaternary carbons. The

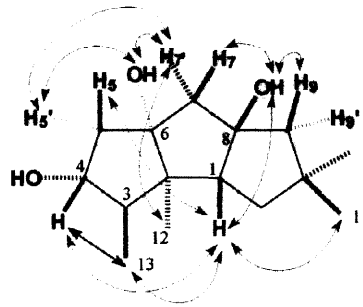
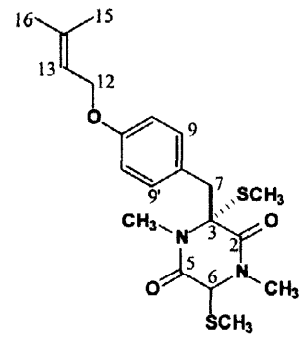


Figure 3. NOESY correlation for *ent*-gloeosteretriol (**10**) in dioxane-d_4.

^{1}H-^{1}H COSY spectrum showed isolated spin systems consisting of H3-H4-H5, H3-Me13, H1-H11, H7-H7′, and H9-H9′. An HMBC spectrum showed correlations from Me12 to C1, C2, C3 and C6; from H7 to C1, C2, C5, C6 and C8; from H9 to C8, C11, C1 and C10; H_{13}14 and $H_3$15 correlated to each others carbon and to C9, C10 and C11. These HMBC data verified that **10** had the same planar structure as two known compounds: gloeosteretriol (**11**) from *Gloeostereum incarnatum*[13] and arthrosporol (**12**) from an *Arthroconidial* fungus.[14] Correlations observed from a NOESY experiment on **10** in dioxane-d_8 illustrated in Figure 3 indicated that relative stereochemistry of **10** is the same as that of **11**, but the optical rotations are opposite (**10**: $[\alpha]^{25}_D$ = -1.5° (*c*, 1.33, CH_3OH), **11**: $[\alpha]^{22}_D$ = +5.6° (*c*, 0.115, CH_3OH),[13] **12**: $[\alpha]_D$ = -62.1° (*c*, 1.0, CH_3OH) and -29° (*c*, 2.0, $CHCl_3$)[14]). Therefore, the stereochemistry of **10** is assigned as the enantiomer of gloeosteretriol. *Ent*-gloeosteretriol (**10**) was found to be anti-microbial *(Bacillus subtilus)* active.[12]



diketopiperazine **13**: α-6-SMe
6-*Epi*-diketopiperazine **14**: β-6-SMe

During the isolation studies of **A** and **B** category compounds in *C. consors*, two diketopiperazines, **13** and **14**, were also obtained as major components from both salt water and deionized water fermentation of a *C. consors* strain (ATCC #11574). Compound **13** was quickly identified as 3,6-*cis*-dithiomethyl-diketopiperazine, a known diketopiperazine isolated from *Gliocladium deliquescanes*,[15] and **14** was seen to be a diasteromer of **13**. The major ^{1}H NMR shift differences between **13** and **14** are at H6 (δ 4.20 vs. 4.62) and at both SMe3 (δ 2.30 and 1.58) and SMe6 (δ 2.18 and 1.96), respectively. Thus, the structural differences between **13** and **14** can be attributed to a *trans* vs *cis* stereochemical differences between the two SMe groups. When the two SMe groups were *trans* (as compound **14**), a 0.42 ppm downfield shift was observed for H6. Similar data were reported for two de-N-methyl diketopiperazines of **13** and **14** analogues, named Sch54794 (H6 δ 4.25) and Sch54796 (H6 δ 4.93) respectively, isolated from the fungus *Tolypocladium* sp.[16]

Table 3. Summary of sources, cultures and compounds isolated

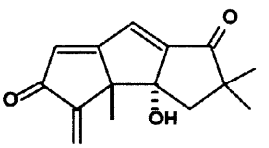
Entry no.	Fungus strain	Source	Broth condition	Compounds isolated
1	92-902	Sponge *J.* cf. *johnstoni*	sw-MEB	**1-5**
2	95-1005C	Sponge *Haliclona* sp	sw-MEB	**6-8, 10**
3	*C. consors* 66132	ATCC	sw-CGB	**9**
4	*C. consors* 66132	ATCC	di-CGB	-
5	*C. consors* 66132	ATCC	sw-MEB	-
6	*C. consors* 66132	ATCC	di-MEB	-
7	*C. consors* 11574	ATCC	sw-CGB	**13, 14**
8	*C. consors* 11574	ATCC	di-CGB	**14**
9	*C. consors* 11574	ATCC	sw-MEB	**13, 14**
10	*C. consors* 11574	ATCC	di-MEB	**13, 14**
11	*C. consors* 20305	ATCC	sw-CGB	-
12	*C. consors* 20305	ATCC	di-CGB	-

* sw = sea water, di = deionized water, MEB = malt extract broth, CGB = coriolus glucose broth.

A summary of our investigation on the variation in metabolite production as a function of fungal sources and broth conditions appears in Table 3. In our original study of the *J.* cf. *johnstoni* derived fungus 92-902 (entry 1) we reported the Cl-containing compounds **3** – **5**[2] as well as coriolin B (**1**) and dihydrocoriolin C (**2**).

The latter two were originally isolated from *Coriolus consors*.[4] Alternatively, *Coriolus consors* from ATCC (strain # 66132 and 11574) grown in sw-MEB conditions (entries 5 and 9) did not give Cl-containing hirsutane derivatives, and the latter afforded diketopiperazines **13** and **14**. The *Haliclona* sp. derived fungus 95-1005C (entry 2) was a source of hirsutane derivatives **6** - **8** and **10** when it was grown in sw-MEB conditions. The ATCC *C. consors* (strain #66132) grown in sw-CGB (entry 3) yielded hirsutanol D (**9**). By contrast no hirsutane derivatives were isolated from other ATCC strains (strain #11574 and 20305) under any conditions. Finally, *C. consors* strain 11574 was a rich source of diketopiperazines **13** and **14** under all culture conditions. The factors controlling these large variations are not clear at this point.

The isoprene metabolites isolated during this work belong to either the hirsutane (**A**) or isohirsutane (**B**) category of sesquiterpenes. Hirsutanols A (**6**) and B (**7**) are structurally similar to incarnal (**15**) isolated from the broth culture of *Gloeostereum incarnatum*, a terrestrial bacterium.[17] More distant from incarnal is hirsutanol C (**8**) which resembles **6** except for the saturation of C13 in the β position. Hirsutanol D (**9**) is somewhat unique in comparison to other known hirsutane derivatives,[1] as it possesses the new isohirsutane **B** frame. Hirsutanol D (**9**) is the 7-epimer of cucumin F, a sesquiterpenoid from *Macrocystidia cucumis*.[18] It is also interesting that our unidentified marine-derived fungi produced **10**, the enantiomer of gloeosteretriol (**11**).



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EXPERIMENTAL SECTION

The NMR spectra were recorded at 500 MHz for ^1H and 62.5 and 125 MHz for ^{13}C in CD_3OD. ^1H-^1H COSY, HMQC and HMBC data were measured in CD_3OD; NOESY spectra were measured in dioxane-d_8. FABMS, CIMS and EIMS methods were also used. Optical rotations were determined on a digital polarimeter in methanol. The assignments of ^{13}C and ^1H NMR data were made by using HMQC data to determine one bond H-C connectivities, HMBC data to determine two or three bond H-C connectivities, and NOESY data to interrelate protons with close spatial proximity.

Culture of organism. The fungus (coll. no. 95-1005C) was cultured from a common Indo-Pacific sponge, *Haliclona* sp. The culture was aseptically collected from the interior of the sponge sample and plated onto Difco's corn meal agar (17 g/L) made with filtered (0.2 μm) Monterey Bay sea water, and supplemented with 100 mg/L each of penicillin and streptomycin to decrease bacterial contamination. This fungus was chosen for further study because the ethyl acetate extract of a 125 mL test broth showed selective cytotoxicity.[11] The 125 mL screening culture and the 8 liter culture were grown on a rotary shaker at 27°C at 180 rpm for three weeks.

Three different strains of *Coriolus consors* (strain #66132, 11574 and 20305) were obtained from the ATCC list and cultured in two different media conditions, malt extract broth (MEB) in which 15 g/L of malt extract (DIFCO) dissolved in filtered (0.2μm) Monterey Bay sea water, and coriolus glucose broth (CGB) described by Takeuchi, et. al.[19] The disk diffusion assay is used extensively to test susceptibility of microbes to known antimicrobials such as penicillin and ampicillin. We used Mueller-Hinton agar as the medium and *Bacillus subtilus* for Gram positive bacteria. Both hirsutanol A and *ent*-gloeosteretriol were found to be active against *B. subtilus* at 200 μg/disc, 20% of positive control, tetracycline.

Extraction and isolation of hirsutanol A (6), hirsutanol B (7), hirsutanol C (8), and *ent*-gloeosteretriol (10) from fungus 95-1005C. The mycelium and broth from 8 liters of cultured fungi were separated by filtration and each was extracted independently. The broth was extracted with ethyl acetate. The extract (1.36g) was partitioned between 10% aqueous methanol and hexane twice. The aqueous methanol

soluble portion was further partitioned between 50% aqueous methanol and methylene chloride twice. The 50% aqueous methanol solubles (508 mg) were chromatographed on sephadex eluted with methanol. Fractions 6 and 7 were further separated with sephadex eluted with 50:50 MeOH:CH_2Cl_2. Fractions 10-12 were purified with reversed phase HPLC (eluted by gradient with methanol-water; 30:70 to 100:0) to yield compounds 6 (25.6 mg), 7 (2.5 mg), 8 (8.0 mg), and 10 (20.0 mg).

Isolation of hirsutanol D (9) from cultured *Coriolus consors* (strain #66132). ATCC Strain #66132 was grown in 0.5 L CGB salt water, and the mycelium was separated by vacuum filtration from the cultured fungus broth. The concentrated ethyl acetate extract of the liquid broth provided 68 mg of oil which was subjected to normal phase HPLC (3% MeOH-CH_2Cl_2) and **9** (1.3 mg) was isolated in the fourth fraction.

Isolation of diketopiperazines (13 and 14) from *Coriolus consors* (strain #11574): ATCC strain #11574 was grown in fifteen 1 L erlenmeyer flasks (500 mL broth/flask) in salt water MEB broth conditions. The ethyl acetate extract of the broth was partitioned between aqueous methanol with hexane, methylene chloride. The 0.52 g CH_2Cl_2 extract was separated by reversed phase flash chromatography followed by normal phase HPLC (1% MeOH-CH_2Cl_2) to provide compounds **13** and **14**.

Hirsutanol A (6). $[\alpha]^{25}_D$ = -23.5° (*c*, 0.97, CH_3OH). UV(CH_3OH): 306 (4276), 218 (2920). IR (KBr) ν 3401, 2966, 1678, 1642, 1590, 1455, 1366, 1284, 1155, 1131, 1031, 879. ^{13}C and ^1H NMR data is given in Tables 1 and 2. ^1H NMR (500 MHz, dioxane-d_8) δ 5.97 (s, H5), 6.36 (d, *J* = 2.5 Hz, H7), 4.56 (br-s, H9), 2.18 (d, *J* = 14.5 Hz, H11), 1.63 (d, *J* = 14.5 Hz, H11′), 1.19 (s, $H_3$12), 5.87 (d, *J* = 1.0 Hz, H13), 5.09 (d, *J* = 1.0 Hz, H13′), 0.89 (s, $H_3$14), 1.24 (s, $H_3$15), 3.30 (s, OH-1), 3.96 (br-d, *J* = 2.5 Hz, OH-9).

Hirsutanol B (7). ^1H and ^{13}C NMR data is given in Tables 1 and 2. **7** decomposed in CD_3OD for four days, but compound **6** was stable under such condition. So no optical rotation, UV and IR were available for **7**.

Hirsutanol C (8). $[\alpha]^{25}_D$ = +20.6° (*c*, 0.31, CH_3OH). UV(CH_3OH): 282 (1658), 206 (896). IR (KBr) ν 3367, 2962, 2858, 1686, 1671, 1611, 1466, 1377, 1167, 1077, 1062, 883. ^{13}C and ^1H NMR data is given in Tables 1 and 2. ^1H NMR (500 MHz, dioxane-d_8) δ 2.73 (q, *J* = 7.0 Hz, H3), 5.72 (s, H5), 6.32 (d, *J* = 2.5 Hz, H7), 4.56 (br-s, H9), 2.03 (d, *J* = 14.5 Hz, H11), 1.53 (d, *J* = 14.5 Hz, H11′), 0.97 (s, $H_3$12), 1.00 (d, *J* = 7.0 Hz, $H_3$13), 0.87 (s, $H_3$14), 1.25 (s, $H_3$15), 3.65 (br-s, OH1), 3.87 (br-s, OH9).

Hirsutanol D (9). $[\alpha]^{25}_D$ = -36° (*c*, 0.13, $CHCl_3$). UV($CHCl_3$): 248 (2316). IR (KBr) ν 3413, 2954, 2919, 2849, 1737, 1707, 1655, 1455, 1414, 1378, 1255, 1090, 1014, 861, 797. ^{13}C and ^1H NMR data is given in Tables 1 and 2.

***Ent*-gloeosteretriol (10).** $[\alpha]^{25}_D$ = -1.5° (*c*, 1.33, CH_3OH). UV(CH_3OH): 208 (321). IR (KBr) ν 3426, 3337, 3247, 2948, 2868, 1461, 1382, 1302, 1157, 1087, 1047, 978. ^{13}C and ^1H NMR data is given in Tables 1 and 2. ^1H NMR (500 MHz, dioxane-d_8) δ 2.24 (t, *J* = 10.0 Hz, H1), 1.52 (m, H3), 3.83 (dt, *J* = 8.0, 8.0 Hz, H4), 2.59 (dd, *J* = 8.0, 14.0 Hz, H5), 1.48 (dd, *J* = 8.5, 14.0 Hz, H5′), 1.86 (d, *J* = 13.5 Hz, H7), 1.72 (d, *J* = 13.5 Hz, H7′), 1.55 (d, *J* = 13.5 Hz, H9), 1.44 (d, *J* = 13.5 Hz, H9′), 1.385 (dd, *J* = 10.0, 13.5 Hz, H11), 1.375 (dd, *J* = 10.0, 13.5, H11′), 0.71 (s, $H_3$12), 0.90 (s, $H_3$13), 0.99 (s, $H_3$14), 1.09 (s, $H_3$15), 3.22 (br-s, OH4), 2.99 (s, OH8), 3.07 (br-s, OH6).

***Epi*-diketopiperazine (14):** $[\alpha]^{25}_D$ = +14.7° (*c* 2.2, CH_2Cl_2). ^1H NMR (500 MHz, $CDCl_3$) δ 4.62 (s, H6), 3.66 (d, *J* = 14.0 Hz, H7), 3.07 (d, *J* = 14.0 Hz, H7′), 7.06 (d, *J* = 8.5 Hz, H9, 9′), 6.79 (d, *J* = 8.5 Hz, H10, 10′), 4.44 (d, *J* = 6.0 Hz, $H_2$12), 5.45 (br-t, *J* = 6.5 Hz, H13), 1.72 (s, $H_3$15), 1.77 (s, $H_3$16), 3.05 (s, $NCH_3$1), 1.96 (s, $SCH_3$6), 3.25 (s, $NCH_3$4), 1.58 (s, $SCH_3$3). ^{13}C NMR (125 MHz, $CDCl_3$) δ 164.5 (C2), 76.0 (C3), 164.3 (C5), 65.3 (C6), 40.2 (C7), 125.9 (C8), 131.2 (C9, 9′), 114.8 (C10, 10′), 158.2 (C11), 64.5 (C12), 119.3 (C13), 137.9 (C14), 17.9 (C15), 25.5 (C16), 33.1 (NMe1), 30.3 (NMe4), 13.2 (SMe3), 12.3 (SMe6).

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