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ORIGINAL ARTICLE

Structure elucidation of meroterpenoid habiterpenol, a novel abrogator of bleomycin-induced G2 arrest in Jurkat cells, produced by *Phytohabitans suffuscus* 3787_5

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A novel abrogator of bleomycin-induced G2 arrest in Jurkat cells, habiterpenol (1), was isolated from the culture broth of *Phytohabitans suffuscus* 3787_5. The planar structure of 1 was elucidated by spectroscopic study (1D and 2D NMR, MS, UV and IR), and the relative stereochemistry was elucidated by ROESY experiments. Compound 1 belongs to a pentacyclic meroterpenoid having a labdan-type diterpene connecting to an indane moiety.
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INTRODUCTION

During the course of screening for microbial abrogators of bleomycin-induced G2 arrest in Jurkat cells, a new compound, named habiterpenol (**1**, Figure 1), was isolated from the culture broth of *Phytohabitans suffuscus* 3787_5. The taxonomy of the producing actinomycete, fermentation, isolation and biological properties of **1** are described in the accompanying paper.[1] In this study, the physicochemical properties and structure elucidation of **1** are described.

RESULTS

Physicochemical properties of habiterpenol

The physicochemical properties of habiterpenol (**1**) are summarized in Table 1. In UV spectra, **1** showed absorption maxima at 221, 234(sh) and 284 nm. In IR spectra, broad OH absorption near 3428 cm^{-1}, typical C–H (CH_2) stretching absorptions at 2929 and 2851 cm^{-1} and aromatic C–C stretch absorptions (for carbon–carbon bonds in the aromatic ring) at 1614 and 1461 cm^{-1} were observed.

Planar structure of habiterpenol

Compound **1** showed a molecular ion peak at *m/z* 366 $[M]^+$ in EI-MS, and the molecular formula $C_{26}H_{38}O$ was assigned on the basis of its HR-EIMS (*m/z* 366.2929 $[M]^+$, $\Delta + 0.6$ mmu), indicating 8 degrees of unsaturation. The ^1H and ^{13}C NMR spectra of **1** in $CDCl_3$ (Table 2) showed 38 proton and 26 carbon signals, which were confirmed by analysis of 2D NMR correlations. The multiplicity of the carbon signals was classified into five methyl carbons, eight sp^3 methylene carbons, three sp^2 methine carbons, three sp^3 methine carbons, two sp^2 quaternary carbons, four sp^3 quaternary carbons and one sp^2 oxygenated quaternary carbon by analysis of DEPT and HMQC data. The connectivity of proton and carbon atoms was established by HMQC (Table 2). As shown in Figure 2, the partial structures I–V were elucidated by ^1H–^1H COSY spectra and TOCSY.

The ^{13}C–^1H long-range couplings of ^2J and ^3J in the HMBC spectra (Figure 3) proved the presence of the following linkages: (1) The cross peaks from the sp^3 methine proton H-10 (δ 0.82) to the sp^3 quaternary carbon C-11 (δ 37.4) and the methyl carbon C-18 (δ 16.4), from sp^3 methylene protons H_2-13 (δ 1.60, 1.40) to the sp^3 quaternary carbon C-15 (δ 33.3), from the methyl protons H_3-18 (δ 0.73) to the sp^3 methine carbon C-6 (δ 57.9), C-11 and the sp^3 methylene carbon C-12 (δ 40.1), and from the methyl protons H_3-19 (δ 0.78) and H_3-20 (δ 0.85) to the sp^3 methine carbon C-10 (δ 56.8) and the sp^3 methylene carbon C-14 (δ 42.2) and C-15 suggested the presence of a 11,15,15-trimethyl-10,11-disubstituted cyclohexane (ring A) containing the partial structure I. (2) Long-range couplings from the sp^3 methylene protons H_2-8 (δ 1.82, 1.02) to the quaternary carbon C-7 (δ 37.4) and C-10, from the methyl protons H_3-17 (δ 0.33) to C-6, C-7 and sp^3 methylene carbon C-8 (δ 43.0), and from H_3-18 to C-6 showed that a 7,11-dimethyl-6,7,10,11-tetrasubstituted cyclohexane ring (ring B) containing the partial structure II is attached to ring A, revealing the presence of a 7,11,15,15-tetramethyl bicyclo[4.4.0]decane moiety. (3) Long-range couplings from the sp^3 methylene protons H_2-1 (δ 2.94, 2.58) to the sp^3 quaternary carbon C-3 (δ 46.3), from the sp^3 methine proton H-2 (δ 1.72) to C-3, C-7 and the methyl carbon C-16 (δ 33.5), from the sp^3 methylene protons H_2-4 (δ 2.25, 1.56) to the sp^3 methine carbon C-2 (δ 63.1), C-3 and C-16, from the sp^3 methylene protons H_2-5 (δ 1.47, 1.15) to C-3 and C-7, from the methyl protons H_3-16 (δ 1.06) to C-2, C-3 and the sp3 methylene carbon C-4 (δ 34.9), and from H_3-17 to C-2 showed that a 3,7-dimethyl-2,3,6,7-tetrasubstituted cyclohexane ring (ring C)

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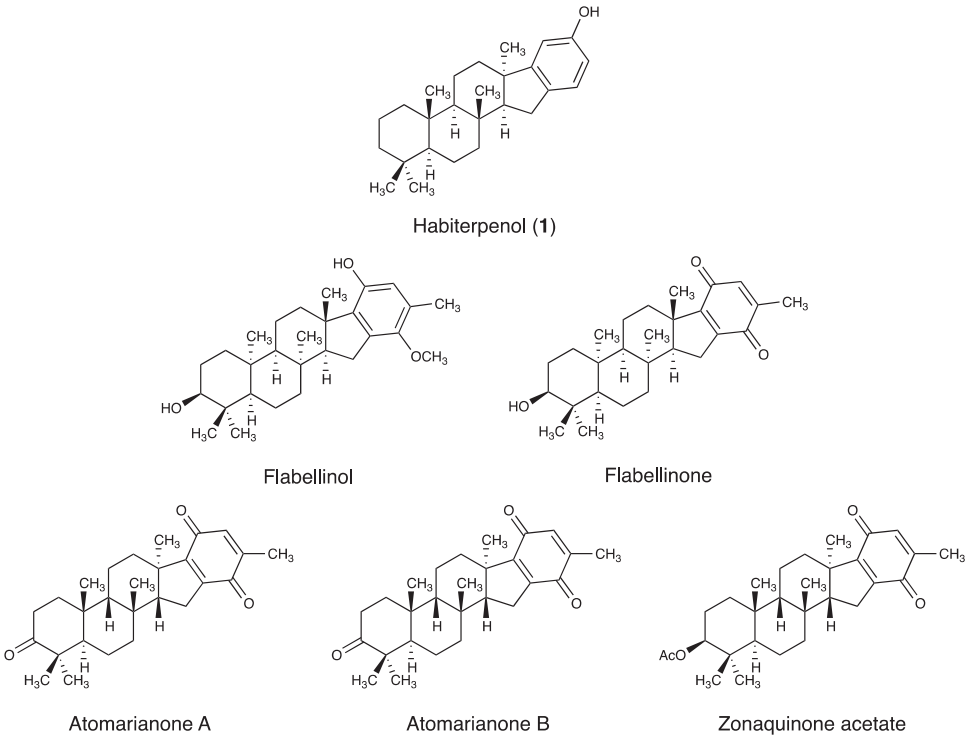

Figure 1 Structures of habiterpenol (**1**) and structurally related compounds.

Table 1 Physicochemical properties of Habiterpenol (1)

Appearance	*Brown powder*
Molecular weight	366
Molecular formula	$C_{26}H_{38}O$
HRFAB-MS	
Calcd	366.2923 $[M]^+$
Found	366.2929 $[M]^+$
UV λ_{max} nm (ε) in MeOH	221 (8050), 234 (3290, sh), 284 (3590)
IR ν_{max} cm^{-1} (KBr)	3509, 3428, 2929, 2851, 1711, 1614, 1461, 1380, 1338, 1264, 1185
$[\alpha]_D^{26}$	-41.6° (*c* 0.1, CH_3OH)
Solubility	
Soluble	$CHCl_3$, MeOH, EtOAc, Acetone
Insoluble	Hexane, H_2O

containing the partial structures III and IV is attached to ring B to form a labdane-type diterpene substructure. (4) Long-range couplings from the methine proton H-2′ (δ 6.96) to the oxygenated sp^2 quaternary carbon C-4′ (δ 154.2) and the sp^2 quaternary carbon C-6′ (δ 153.8), from the sp^2 methine proton H-3′ (δ 6.55) to C-4′, the sp^2 methine carbon C-5′ (δ 107.8) and the sp^2 quaternary carbon C-1′ (δ 135.9), and from the sp^2 methine proton H-5′ (δ 6.54) to the sp^2 methine carbon C-3′ (δ 112.6), C-4′ and C-6′ showed the presence of a 4′-oxy-1′,6′-disubstituted benzene ring (ring E) containing the partial structure V. Further, the two *ortho*-coupling constants (7.5 Hz) observed between aromatic protons H-2′ and H-3′ also supported that they are in the *ortho* position of the benzene ring. (5) This benzene ring (ring E) was connected to a labdane-type diterpene moiety by observation of the ^2J and ^3J cross peaks from H_2-1 to C-1′ and the sp^2 methine carbon C-2′ (δ 124.6), from H-2 to C-1′ and C-6′, from H-2′ to C-1 (δ 30.9), from H-5′ to C-3, and from H_3-16 to C-6′ in HMBC experiments, indicating the presence of an indane moiety (Figure 3). Furthermore, the chemical shifts of C-4′ (δ 154.2) indicated that a hydroxyl group is attached to C-4′. Taking these observations into consideration, the planar structure of **1** was elucidated as shown in Figure 1, which fulfilled the molecular formula and the degrees of unsaturation.

Relative stereochemistry of habiterpenol

Compound **1** has six chiral carbons. The relative stereochemistry of **1** was elucidated by analysis of ROESY experiments as shown in Figure 4. NOEs were observed between H_3-18 (δ 0.73) and H_3-19 (δ 0.78), between H_{ax}-13 (δ 1.60) and H_3-19 and between H_{ax}-13 and H_3-18, indicating that they are all oriented to the same molecular face. On the other hand, NOEs were observed between H-10 (δ 0.82) and H_{ax}-14 (δ 1.15) and between H_{ax}-12 (δ 0.84) and H_{ax}-14, indicating that they are all oriented to the opposite molecular face. These results strongly suggested *trans*-diaxal disposition of H-10 and H_3-18. NOEs were also observed between H_{ax}-9 (δ 1.26) and H_3-17 (δ 0.33), between H_{ax}-9 and H_3-18 and between H_3-17 and H_3-18, indicating that they are all oriented to the same molecular face. On the other hand, NOEs were observed between H-6 (δ 0.85) and H_{ax}-8 (δ 1.02) and between H_{ax}-8 and H-10, indicating that they are all oriented to the opposite molecular face. These data were consistent with *trans* disposition of H-6 and H_3-17. Furthermore, NOEs were observed between H-2 (δ 1.72) and H-6, between H-2 and H_3-16 (δ 1.06) and between H_3-17 and H_{eq}-1 (δ 2.58), indicating that the C/D ring junction is *cis*. This *cis* junction was also supported by the fact that the chemical shift of methyl proton H_3-17 was shifted upfield due to the anisotropic effect of the hydroxyphenyl group. Taken together, these findings suggested that **1** has a *trans–transoid–trans–transoid–cis*

Table 2 ^{1}H and ^{13}C NMR chemical shifts of habiterpenol (1) in $CDCl_3$

Position	δ_C[a]	*Mult.*	δ_H[b]	*Mult.*	J *(Hz)*
1	30.9	t	2.94	ddd	16.0, 7.0, 1.0
			2.58	d	16.0
2	63.1	d	1.72	br. d	7.0
3	46.3	s			
4	34.9	t	2.25	dt	14.0, 4.0
			1.56	m	
5	18.2	t	1.47	m	
			1.15	m	
6	57.9	d	0.85	s	
7	37.4	s[c]			
8	43.0	t	1.82	dt	13.0, 3.0
			1.02	dt	13.0, 4.0
9	18.4	t	1.47	m	
			1.26	m	
10	56.8	d	0.82	m	
11	37.4	s[c]			
12	40.1	t	1.72	m	
			0.84	dt	11.5, 2.5
13	18.6	t	1.60	m	
			1.40	m	
14	42.2	t	1.36	m	
			1.15	m	
15	33.3	s			
16	33.5	q	1.06	3H, s	
17	16.43	q[d]	0.33	3H, s	
18	16.42	q[d]	0.73	3H, s	
19	21.5	q	0.78	3H, s	
20	33.4	q	0.85	3H, s	
1'	135.9	s			
2'	124.6	d	6.96	br. d	7.5
3'	112.6	d	6.55	dd	7.5, 2.5
4'	154.2	s			
5'	107.8	d	6.54	s	
6'	153.8	s			
4'-OH			4.67	br. s	

[a]^{13}C chemical shifts are shown in δ values (p.p.m.) relative to $CDCl_3$ at 77.0 p.p.m.
[b]^{1}H chemical shifts are shown in δ values (p.p.m.) relative to $CDCl_3$ at 7.26 p.p.m. followed by multiplicity and coupling constants (*J* Hz).
[c]Overlapped by other signals (assignment based on HMQC experiments).
[d]Exchangeable signals.

arrangement for the A–B–C–D ring system. Thus, the relative stereochemistry of **1** was presumed to be 2*S**3*R**6*R**7*R**10*S**11*S**.

DISCUSSION

In the course of screening for microbial abrogators of bleomycin-induced G2 arrest in Jurkat cells, habiterpenol (**1**, Figure 1) was isolated from the culture broth of *P. suffuscus* 3787_5.[1] In this study, the structure of **1** was elucidated to be a meroditerpenoid with a labdane-type diterpene connecting an indan moiety. A number of meroditerpenes having a benzofuro- or benzopyrano-ring moiety were isolated from natural sources, but five kinds of indan like moiety-containing meroterpenoids including **1** (Figure 1) have been reported so far. Sabry *et al.*[2] isolated flabellinol and flabellinone from *Stypopodium flabelliforme* as potent inhibitors of the sodium channel. Abatis *et al.*[3] isolated atomarianones A and B from *Taonia atomari* as cytotoxic agents in two lung cancer cell lines. Penicooke *et al.*[4] isolated zonaquinone acetate from *S. zonale* as a cytotoxic agent in

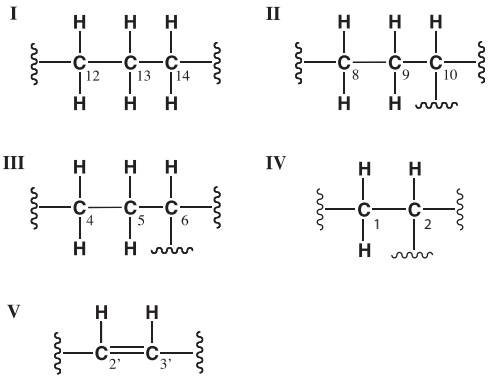

Figure 2 Partial structures of **1** elucidated by ^{1}H–^{1}H COSY and TOCSY.

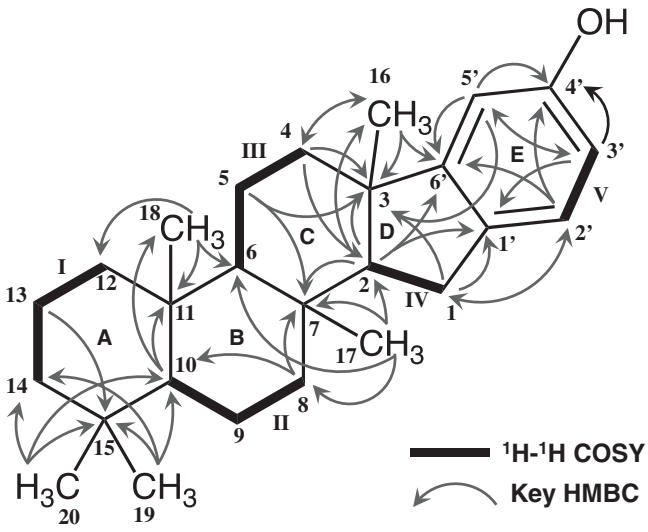

Figure 3 Planar structure of **1** elucidated by HMBC experiments.

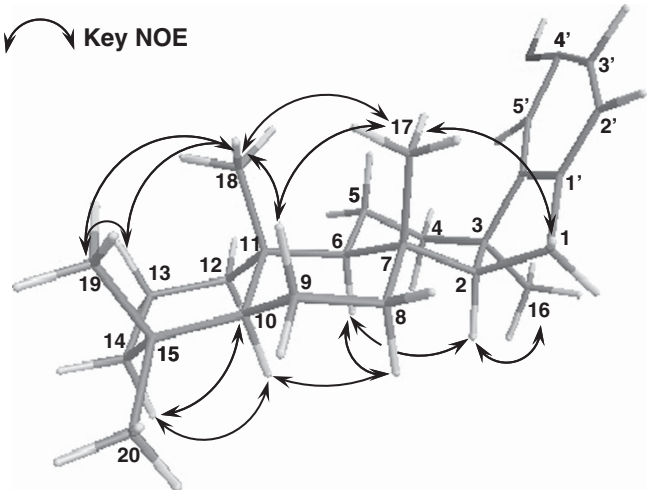

Figure 4 Relative configuration of **1** elucidated by NOE experiments. A full color version of this figure is available at *The Journal of Antibiotics* journal online.

breast cancer and colon cancer cell lines. Interestingly, these meroditerpenoids were isolated from marine algae, while **1** was isolated from an actinomycete. The stereochemistries of these five

meroditerpenoids differed as follows (Figure 1). Flabellinol and flabellinone have a unique *cis–cisoid–cis–cisoid–trans* A–B–C–D ring structure that induces rings A and C into chair conformations and ring B into a twist boat.[2] Atomarianone A and zonaquinone acetate have an unprecedented *trans–cisoid–cis–cisoid–trans* A–B–C–D ring structure, while atomarianone B is the epimer of atomarianone A at C-7 constructing a *trans–cisoid–trans–transoid–trans* A–B–C–D ring structure.[3] As elucidated in this study, **1** has a *trans–transoid–trans–transoid–cis* A–B–C–D ring structure that induces rings A, B and C into chair conformations; therefore, **1** has a unique ring structure among diterpens fused with an indane moiety in natural products. Further experiments, for example, X-ray crystallography or total synthesis, are needed to confirm the absolute stereochemistry of **1**.

METHODS

General

Various NMR spectra were obtained using an Agilent Technologies XL-400 (400 MHz) spectrometer (Agilent Technologies, Santa Clara, CA, USA). Electrospray ionization mass spectrometry (ESI-MS) was conducted on a JEOL JMS-T100LP spectrometer (Tokyo, Japan). UV–visible and IR spectra were measured with a Beckman DU640 spectrophotometer (Beckman Coulter, Inc., Fullerton, CA, USA) and a Horiba FT-210 Fourier transform infrared spectrometer (Horiba, Kyoto, Japan), respectively. Optical rotations was recorded on a JASCO model DIP-181 polarimeter (JASCO, Tokyo, Japan).

Producing actinomycete *P. suffuscus* 3787_5 and isolation of habiterpenol

The fermentation and purification procedures of **1** were described in the accompanying paper.[1] The habiterpenol-producing actinomyces *P. suffuscus* 3787_5 was isolated from a soil sample collected on Ishigaki Island, Japan. Bioassay-guided fractionation of the culture broth of the actinomycete, including organic solvent extraction, ODS gel column chromatography and reversed phase (C4) HPLC, yielded **1**.

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1 Uchida, R. *et al.* Habiterpenol, a novel abrogator of bleomycin-induced G2 arrest in Jurkat cells, produced by *Phytohabitans suffuscus* 3787_5. *J. Antibiot.* **67,** 777–781 (2014).
2 Sabry, O. M. *et al.* Neurotoxic meroditerpenoids from the tropical marine brown alga *Stypopodium flabelliforme*. *J. Nat. Prod.* **68,** 1022–1030 (2005).
3 Abatis, D. *et al.* Atomarianones A and B: two cytotoxic meroditerpenes from the brown alga *Taonia atomaria*. *Tetrahedron Lett.* **46,** 8525–8529 (2005).
4 Penicooke, N. *et al.* Antiproliferative activity and absolute configuration of zonaquinone acetate from the Jamaican alga *Stypopodium zonale*. *Phytochemistry* **87,** 96–101 (2013).

Supplementary Information accompanies the paper on The Journal of Antibiotics website (http://www.nature.com/ja)