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Cell-adhesion Inhibitors Produced by a Sea Hare-derived *Periconia* sp. II

Absolute Stereostructures of Peribysins H and I

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Abstract Peribysins H (**1**) and I (**2**) have been isolated from a strain of *Periconia byssoides* originally separated from the sea hare *Aplysia kurodai*. Their absolute stereostructures have been elucidated on the basis of spectroscopic analyses using 1D and 2D NMR techniques and some chemical transformations including the modified Mosher's method. These metabolites inhibited the adhesion of human-leukemia HL-60 cells to HUVEC.

Keywords fungus, peribysins, cell-adhesion inhibitor, *Periconia* sp., sea hare

Introduction

Based on the fact that some of the bioactive materials isolated from marine animals have been produced by bacteria, we have focused our attention on new antitumour materials from microorganisms inhabiting the marine environment [1~3]. As part of this study, we have previously isolated the cell-adhesion inhibitors, macrosphelides E~I, L [4, 5] and peribysins A~G [6, 7], from a strain of *Periconia byssoides* OUPS-N133 originally separated from the sea hare *Aplysia kurodai*. All of these compounds except for macrosphelide I inhibited the adhesion of human-leukemia HL-60 cells to human-umbilical-vein endothelial cells (HUVEC) more potently than herbimycin A [8, 9]. Further investigation of the metabolites from this fungal strain has now led to the isolation of additional new anti-adhesion compounds designated peribysins H (**1**) and I (**2**) (Fig. 1). We describe herein the absolute stereostructures of peribysins H (**1**) and I (**2**) (Fig. 1) in addition to their inhibition of cell adhesion.

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Materials and Methods

General

UV spectra were recorded on a Shimadzu spectrophotometer and IR spectra on a Perkin Elmer FT-IR spectrometer 1720X. NMR spectra were recorded at 27°C on Varian UNITY INOVA-500 and MERCURY spectrometers with tetramethylsilane (TMS) as an internal reference. EIMS was determined using a Hitachi M-4000H mass spectrometer. Optical rotations were recorded on a JASCO J-820 polarimeters. Liquid chromatography over silica gel (mesh 230~400) was performed at medium pressure. HPLC was run on a Waters ALC-200 instrument equipped with a differential refractometer (R 401) and Shim-pack PREP-ODS (25 cm×20 mm i.d.). Analytical TLC was performed on precoated Merck aluminium sheets (DC-Alufolien Kieselgel 60 F254, 0.2 mm) with the solvent system CH_2Cl_2-MeOH (9 : 1), and compounds were

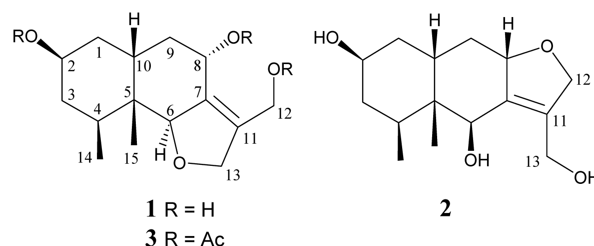

Fig. 1 Structures of peribysin H (**1**) and I (**2**).

viewed under UV lamp and sprayed with 10% H_2SO_4 followed by heating.

Culturing and Isolation of Metabolites

A strain of *Periconia byssoides* OUPS-N133, separated from the sea hare *Aplysia kurodai*, was cultured at 27°C for four weeks in a liquid medium (90 liters) containing malt extract 1.0%, glucose 1.0% and peptone 0.05% in artificial seawater adjusted to pH 7.5. As reported previously [4], the AcOEt extract (5.7 g) of the culture filtrate was successively chromatographed on Sephadex LH-20 (CH_2Cl_2 - MeOH, 1 : 1) and silica gel (CH_2Cl_2/MeOH). The MeOH - CH_2Cl_2 (5 : 95) eluate (214.6 mg) from silica gel column chromatography was purified by HPLC using MeOH - H_2O (7 : 3) as the eluent to afford **1** (7.8 mg, 0.14%) and **2** (10.2 mg, 0.18%).

Acetylation of Peribysin H (1)

To a solution of **1** (2.5 mg) in pyridine (1.0 ml) was added Ac_2O (1.0 ml), and the reaction mixture was left at room temperature overnight. The mixture was concentrated to dryness under reduced pressure, and residue was purified by HPLC using MeOH - H_2O (9 : 1) as the eluent to afford triacetate **3** (1.5 mg) as a pale yellow oil.

Triacetate **3**: EIMS *m/z* 394 (M^+, 1.5%); HREIMS *m/z* for $C_{21}H_{30}O_7$ (M^+), Calcd: 394.2103; Found: 394.2101; ^1H NMR δ ppm ($CDCl_3$): 0.79 (3H, s, 15-H), 1.06 (3H, d, J=7.5 Hz, 14-H), 1.65 (1H, m, 3β-H), 1.67 (1H, m, 9α-H), 1.68 (1H, m, 1β-H), 2.02 (1H, m, 4-H), 2.02 (1H, m, 9β-H), 2.03 (1H, m, 10-H), 2.03 (3H, s, $COOCH_3$), 2.03 (1H, m, 3α-H), 2.04 (3H, s, $COOCH_3$), 2.06 (3H, s, $COOCH_3$), 2.08 (1H, m, 1α-H), 4.56 (1H, dt, J=13.4, 6.8 Hz, 13A-H), 4.65 (1H, d, J=13.4 Hz, 13B-H), 4.65 (1H, d, J=13.2 Hz, 12A-H), 5.03 (1H, d, J=13.2 Hz, 12B-H), 5.04 (1H, m, 2-H), 5.12 (1H, br s, 6-H), 5.78 (1H, dd, J=10.8, 3.2 Hz, 8-H).

Formation of the (*R*)- and (*S*)-MTPA Esters 4a and 4b from Peribysin H (1)

(*R*)-MTPA (2.0 mg), dicyclohexylcarbodiimide (DCC) (2.0 mg) and 4-(dimethylamino)-pyridine (DMAP) (1.0 mg) were added to a CH_2Cl_2 solution (0.2 ml) of peribysin H (**1**) (0.8 mg), and the reaction mixture was left at room temperature for 3 hours. The solvent was evaporated off under reduced pressure, and the residue was purified by HPLC using MeOH - H_2O (9 : 1) as the eluent to afford (*R*)-MTPA ester **4a** (0.7 mg) as an amorphous powder. The same reaction with **1** (0.7 mg) using (*S*)-MTPA (2.1 mg) gave ester **4b** (0.7 mg).

Ester **4a**: EIMS *m/z* 700 (M^+, 0.6%); HREIMS *m/z* for $C_{35}H_{38}F_6O_8$ (M^+), Calcd: 700.2460; Found: 700.2458; ^1H NMR δ ppm ($CDCl_3$): 0.73 (3H, s, 15-H), 1.07 (3H, d, J=7.3 Hz, 14-H), 1.34 (1H, dt, J=14.8, 4.8 Hz, 1β-H), 1.43 (1H, dt, J=14.8, 4.8 Hz, 3β-H), 1.57 (1H, m, 1α-H), 1.65 (1H, m, 9α-H), 1.69 (1H, m, 3α-H), 1.81 (1H, m, 4-H), 2.02 (1H, m, 10-H), 2.12 (1H, dd, J=14.3, 5.0 Hz, 9β-H), 3.55 (3H, s, OCH_3), 3.56 (3H, s, OCH_3), 3.85 (1H, m, 2-H), 4.41 (1H, dd, J=13.2, 6.0 Hz, 13A-H), 4.55 (1H, dd, J=13.2, 3.9 Hz, 13B-H), 4.59 (1H, br s, 6-H), 4.78 (1H, d, J=12.5 Hz, 12A-H), 5.55 (1H, d, J=12.5 Hz, 12B-H), 5.95 (1H, dd, J=5.0, 2.9 Hz, 8-H), 7.40 (8H, m, Ar.H) and 7.50 (2H, m, Ar.H).

Ester **4b**: EIMS *m/z* 700 (M^+, 0.8%); HREIMS *m/z* for $C_{35}H_{38}F_6O_8$ (M^+), Calcd: 700.2460; Found: 700.2459; ^1H NMR δ ppm ($CDCl_3$): 0.73 (3H, s, 15-H), 1.07 (3H, d, J=7.3 Hz, 14-H), 1.30 (1H, dt, J=14.8, 4.8 Hz, 1β-H), 1.46 (1H, dt, J=14.8, 4.8 Hz, 3β-H), 1.57 (1H, m, 1α-H), 1.58 (1H, m, 9α-H), 1.73 (1H, m, 3α-H), 1.88 (1H, m, 4-H), 1.96 (1H, m, 10-H), 2.04 (1H, dd, J=14.3, 5.1 Hz, 9β-H), 3.44 (3H, s, OCH_3), 3.51 (3H, s, OCH_3), 3.85 (1H, m, 2-H), 4.57 (1H, dd, J=13.2, 6.0 Hz, 13A-H), 4.65 (1H, dd, J=13.2, 3.9 Hz, 13B-H), 4.80 (1H, d, J=12.5 Hz, 12A-H), 4.89 (1H, br s, 6-H), 5.47 (1H, d, J=12.5 Hz, 12B-H), 6.00 (1H, dd, J=5.1, 3.0 Hz, 8-H), 7.41 (8H, m, Ar.H) and 7.50 (2H, m, Ar.H).

Formation of the (*R*)- and (*S*)-MTPA Esters 5a and 5b from Peribysin I (2)

(*R*)-MTPA (2.0 mg), dicyclohexylcarbodiimide (DCC) (2.1 mg) and 4-(dimethylamino)-pyridine (DMAP) (1.1 mg) were added to a CH_2Cl_2 solution (0.2 ml) of peribysin H (**1**) (0.7 mg), and the reaction mixture was left at room temperature for 3 hours. The solvent was evaporated off under reduced pressure, and the residue was purified by HPLC using MeOH - H_2O (9 : 1) as the eluent to afford (*R*)-MTPA ester **5a** (0.7 mg) as an amorphous powder. The same reaction with **1** (0.8 mg) using (*S*)-MTPA (2.1 mg) gave ester **5b** (0.8 mg).

Ester **5a**: EIMS *m/z* 916 (M^+, 0.3%); HREIMS *m/z* for $C_{45}H_{45}F_9O_{10}$ (M^+), Calcd: 916.2857; Found: 916.2860; ^1H NMR δ ppm ($CDCl_3$): 0.77 (3H, d, J=6.6 Hz, 14-H), 0.98 (3H, s, 15-H), 1.36 (1H, q, J=12.2 Hz, 3β-H), 1.69 (1H, m, 3α-H), 1.72 (1H, m, 9α-H), 1.73 (1H, m, 4-H), 1.76 (1H, m, 1-H), 1.88 (1H, m, 9β-H), 1.98 (1H, m, 10-H), 3.50 (3H, s, OCH_3), 3.53 (3H, s, OCH_3), 3.55 (3H, s, OCH_3), 4.30 (1H, m, 8-H), 4.58 (1H, dd, J=12.4, 2.2 Hz, 12A-H), 4.62 (1H, dd, J=12.4, 2.2 Hz, 12B-H), 5.07 (1H, d, J=12.2 Hz, 13A-H), 5.12 (1H, d, J=12.2 Hz, 13B-H), 5.21 (1H, m, 2-H), 5.64 (1H, s, 6-H), 7.40 (10H, m, Ar.H) and 7.50 (5H, m, Ar.H).

Ester **5b**: EIMS *m/z* 916 (M^+, 0.5%); HREIMS *m/z* for $C_{45}H_{40}F_9O_{10}$ (M^+), Calcd: 916.2857; Found: 916.2861; ^1H

Table 1 Physico-chemical properties of peribysin H (**1**) and I (**2**)

		1	**2**
Appearance		Pale yellow oil	Pale yellow oil
$[\alpha]_D^{22}$		−200.2 (*c* 0.28, EtOH)	−34.1 (*c* 0.14, EtOH)
HREIMS	Found:	268.1673 $(M)^+$	268.1674 $(M)^+$
	Calcd:	268.1673 (for $C_{15}H_{24}O_4$)	268.1673 (for $C_{15}H_{24}O_4$)
Molecular formula		$C_{15}H_{24}O_4$	$C_{15}H_{24}O_4$
UV λ_{max} (EtOH) nm (log ε)		223 (2.31)	215 (1.87)
IR ν_{max} (neat) cm^{-1}		3385, 1654	3377, 1649
TLC Rf[a]		0.21	0.18
Solubility	soluble	DMSO, MeOH, acetone	DMSO, MeOH, acetone
	insoluble	CH_2Cl_2, H_2O	CH_2Cl_2, H_2O

[a] Silica gel (10% MeOH in CH_2Cl_2).

NMR δ ppm ($CDCl_3$): 0.73 (3H, d, J=6.6 Hz, 14-H), 0.79 (3H, s, 15-H), 1.39 (1H, q, J=12.2 Hz, 3β-H), 1.50 (1H, m, 4-H), 1.62 (1H, m, 1-H), 1.70 (1H, q, J=12.2 Hz,, 9α-H), 1.71 (1H, m, 3α-H), 1.88 (1H, m, 9β-H), 1.90 (1H, m, 10-H), 3.47 (3H, s, OCH_3), 3.53 (3H, s, OCH_3), 3.55 (3H, s, OCH_3), 4.62 (1H, m, 8-H), 4.68 (1H, dd, J=12.5, 2.2 Hz, 12A-H), 4.71 (1H, dd, J=12.5, 2.2 Hz, 12B-H), 5.05 (1H, d, J=12.2 Hz, 13A-H), 5.12 (1H, d, J=12.2 Hz, 13B-H), 5.12 (1H, m, 2-H), 5.71 (1H, s, 6-H), 7.40 (10H, m, Ar.H) and 7.49 (5H, m, Ar.H).

Cell Adhesion Assay

This assay was carried out according to a modification of the Miki's method using 3-(4,5-di-methyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT)-labeled cells [11]. HUVEC (DIA-IATRON Co., Ltd.) were cultured until confluent in a 96-well plate in medium 199 (Gibco) containing 10% fetal calf serum (FCS, Gibco) and washed with phosphate buffered saline (PBS, DIA-IATRON Co., Ltd.) containing 20% FCS. The HUVEC were stimulated with a solution of lipopolysaccharides (LPS, Sigma) in RPMI 1640 medium (Gibco) containing 10% FCS for 4 hours in the presence of various concentrations of macrosphelides, and then MTT-labeled HL-60 cells were added and incuvated for 40 minutes at 37°C in 5% CO_2. Unbound cells were gently washed out with PBS containing 10% FCS, and DMSO was added to lyse the adherent HL-60 cells. Absorbance at 540 nm was measured using a microplate reader (Model 450, BIO-RAD).

Results and Discussion

The fungal strain was cultured at 27°C for 4 weeks in a medium containing malt extract 1%, glucose 1% and peptone 0.05% in artificial seawater adjusted to pH 7.5, as reported previously [4~7]. The EtOAc extract of the culture filtrate was purified by fractionation employing a combination of Sephadex LH-20 and silica gel column chromatographies and reversed phase HPLC to afford Peribysins H (**1**) and I (**2**). The physico-chemical properties of these compounds are summarized in Table 1.

Peribysin H (**1**) had the molecular formula $C_{15}H_{24}O_4$ established by the $[M]^+$ peak of **1** in high-resolution electron-impact mass spectmetry (HREIMS). Its IR spectrum exhibited bands at 3385 and 1654 cm^{-1}, characteristic of an alcohol and a double bond. A close inspection of the ^1H and ^{13}C NMR spectra of **1** (Table 2) by DEPT and ^1H-^{13}C COSY experiments revealed the presence of one tetrasubstituted double bond (C-7 and C-11), one secondary methyl (C-14), one tertiary methyl (C-15), four sp^3-hybridized methylenes (C-1, C-3, C-9, C-12 and C-13) including two oxymethylenes (C-12 and C-13), five sp^3-methines (C-2, C-4, C-6, C-8 and C-10) including three oxymethines (C-2, C-6 and C-8), one quarternary sp^3-carbon (C-5). The ^1H-^1H COSY analysis of **1** led to a partial structural unit as shown by bold-faced lines in Fig. 2, which was supported by HMBC correlations (Table 2). The connection of this unit and the remaining functional groups was determined on the basis of the key HMBC correlations summarized in Fig. 2. The acetylation of **1** gave triacetate **3**, and proton signals for 2-H, 8-H and H-12 were downfield-shifted from δ_H 3.76, δ_H 4.59, δ_H 4.23 and δ_H 4.33 to δ_H 5.04, δ_H 5.78, δ_H 4.65 and δ_H 5.03, respectively. This fact implied that the hydroxyl groups exist at C-2, C-8 and C-12. Based on the above evidence, the planar structure of **1** was elucidated.

The relative stereochemistry of **1** was deduced from

Table 2 NMR spectral data of peribysin H (**1**) in MeOH-d_4

Position	δ_H[a]	J/Hz	^{1}H-^{1}H COSY	NOE	δ_C	HMBC (C)[b]
1α	1.88 dt	13.2 (1β), 4.1 (2, 10)	1β, 2, 10	1β, 2	38.34 (t)	
β	1.66 ddd	13.2 (1α), 9.4 (2), 3.3 (10)	1α, 2, 10	1α, 3β, 10, 15		2, 5, 9, 10
2	3.76 tt	9.4 (1β, 3β), 4.1 (1α, 3α)	1α, 1β, 3α, 3β	1α, 3α, 4, 9α	68.15 (d)	
3α	1.78 m		2, 3β	2, 3β, 4, 14	39.07 (t)	2, 4, 5, 14
β	1.30 dt	14.7 (3α), 9.4 (2, 4)	2, 3α, 4	1β, 3α, 14, 15		1, 2, 4, 5, 14
4	1.79 m		3β, 14	2, 3α, 6, 9α, 14	36.60 (d)	2, 5, 6, 10, 14, 15
5					43.94 (s)	
6	4.91 br t	3.0 (13α, 13β)	13α, 13β	4, 9α, 12α, 13A, 14	91.83 (d)	4, 7, 11, 15
7					136.99 (s)	
8	4.59 t	6.8 (9α, 9β)	9α, 9β	10, 8-OCH_3	64.34 (d)	6, 7, 9, 10, 11
9α	1.64 ddd	13.5 (9β), 9.2 (10), 6.8 (8)	8, 9β, 10	1, 4, 6, 9β, 13A	36.05 (t)	1, 5, 8, 10
β	1.78 m		8, 9α	9α, 10		1, 5, 7, 8, 10
10	1.58 m		1α, 1β, 9α	1β, 8, 9β, 15	38.52 (d)	1, 8
11					136.27 (s)	
12A	4.23 d	13.8 (12B)	12A	6, 12β	56.35 (t)	7, 11, 13
B	4.33 d	13.8 (12A)	12B	12α, 8-OCH_3		7, 11, 13
13A	4.66 dd	12.2 (13β), 3.0 (6)	6, 13β	6, 9α, 13B	78.28 (t)	6, 7, 11
B	4.70 dd	12.2 (13α), 3.0 (6)	6, 13β	13A		6, 7, 11
14	1.03 d	6.9 (4)	4	3α, 3β, 4, 6, 15	17.84 (q)	3, 4, 5
15	0.80 s			1β, 2β, 10, 14	15.59 (q)	4, 5, 6, 10

[a] ^{1}H chemical shift values (δ ppm from $SiMe_4$) followed by multiplicity and then the coupling constants (J/Hz). Figures in parentheses indicate the proton coupling with that position. [b] Long range ^{1}H-^{13}C correlations from H to C observed in the HMBC experiment.

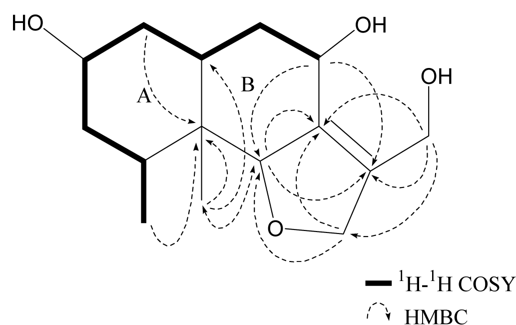

Fig. 2 Selected ^{1}H-^{1}H COSY and HMBC correlations in peribysin H (**1**).

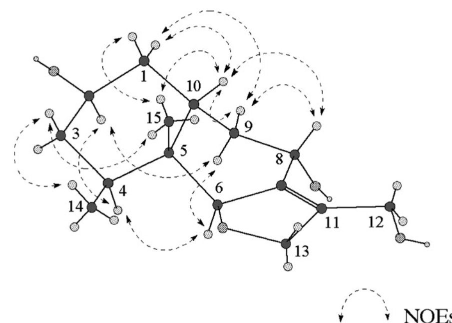

Fig. 3 Observed NOEs for **1**.

NOESY experiments (Fig. 3). NOE correlations from 15-H to 1β-H and 3β-H implied that the A ring exists in a chair conformation with the 5-methyl group, 1β-H and 3β-H in coaxial arrangements. NOEs correlations (15-H/10-H, 14-H/3β-H and 4-H/2-H) suggested that 5-methyl group is oriented *cis* to 10-H, 2-hydroxyl group and 4-methyl group in equatorial arrangements. In addition, NOEs from 6-H to 4-H and 9α-H and from 8-H to 10-H implied that the B ring exists in a twist-boat conformation, and that 6-H is oriented *cis* to both C-4–C-5 bond and 8-hydroxyl group. Based on the evidence summarized above, the relative stereostructure for peribysin H (**1**) was elucidated as depicted in Fig. 3.

The modified Mosher method [10] was applied to determine the absolute configuration of peribysin H (**1**). The ^{1}H chemical-shift differences between the (*R*)- and (*S*)-MTPA esters (**4a** and **4b**) of **1** are shown in Fig. 4. The result suggested that **1** has 8*S* configuration. Hence of the absolute stereostructure of peribysin H (**1**) was determined to be 2*R*, 4*S*, 5*R*, 6*S*, 8*S* and 10*R*.

Peribysin I (**2**) had the same molecular formula as **1**

based on HREIMS. The general features of the ^{1}H and ^{13}C NMR spectra (Table 3) closely resembled those of **1** except that the proton signal for 6-H (δ_H 4.48), 8-H (δ_H 4.98), 10-H (δ_H 2.05), 12A-H (δ_H 4.72), 12B-H (δ_H 4.75), 13A-H (δ_H 4.18) and 13B-H (δ_H 4.25), and the carbon signal for C-6 (δ_C 70.20), C-8 (δ_C 85.37), C-12 (δ_C 77.39) and C-13 (δ_C 56.36) in **2** revealed a chemical shift difference relative to those of **1**. The ^{1}H-^{1}H COSY and HMBC correlations (from 6-H to C-5, C-7, C-8, C-10, C-11 and C-15, from 12-H to C-7 C-8 and C-12, and from 13-H to C-7, C-11 and C-12) led to the planar structure for **2** (Table 3). In NOESY experiments, which cross peaks exhibited a single conformer for **2**, and NOEs (15-H to 1-H, 3β-H, 10-H and 14-H) observed in A ring of **2** were the same as those of peribysin H (**1**), implying that the A ring exists in a chair conformation with 1β-H, 3β-H and 5-methyl group in coaxial arrangements, and with 2- hydroxy group, 10-H and 4-methyl group in equatorial arrangements. In addition, NOEs (10-H to 8-H, 9α-H to 4-H, and 6-H to 4-H, 14-H and 15-H) implied that the B ring exists in a twist-chair conformation with the 8-H, 10-H and 6-hydroxy group in pseud-coaxial arrangements and with 5-methyl group and 6-H in pseud-equatorial arrangements. The above evidence led to the relative sterostructure for peribysin I (**2**).

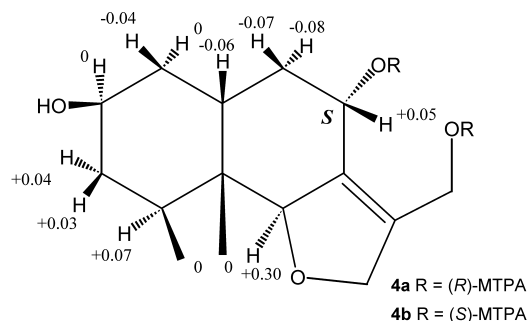

Fig. 4 ^{1}H chemical-shift differences ($\Delta\delta=\delta_S-\delta_R$) between the (*R*)- and (*S*)-MTPA esters (**4a** and **4b**) of peribysin H (**1**).

The modified Mosher method [10] was applied to determine the absolute configuration of peribysin I (**2**). The ^{1}H chemical-shift differences between the (*R*)- and (*S*)-MTPA esters (**5a** and **5b**) of **2** are shown in Fig. 5. The result suggested that **2** has 2*R* configuration, and hence allowed assignment of the other five chiral centers of peribysin I (**2**) as 4*S*, 5*R*, 6*S*, 8*S* and 10*R* configuration.

Peribysins H (**1**) and I (**2**) were evaluated in the adhesion assay system using HL-60 cells and HUVEC, according to

Table 3 NMR spectral data of peribysin I (**2**) in MeOH-d_4

Position	δ_H [a]	*J*/Hz	^{1}H-^{1}H COSY	NOE	δ_C	HMBC (C)[b]
1	1.60 m		2, 10	2, 9β, 10, 15	36.59 (t)	2, 3, 5, 9, 10
2	3.82 tt	12.8 (1, 3β), 5.1 (1, 3α)	1, 3α, 3β	1, 3α, 4, 9α	66.56 (d)	
3α	1.68 ddd	12.8 (3β), 5.1 (2), 2.3 (4)	2, 4	2, 3β, 4, 14	41.18 (t)	1, 2, 4, 5
β	1.29 q	12.8 (2, 3α, 4)	2, 4	3α, 14, 15		2, 4, 14
4	1.74 dqd	12.8 (3β), 6.8 (14), 2.3 (3α)	3α, 3β, 14	2, 3α, 6, 9α, 14	30.55 (d)	5, 10
5					42.10 (s)	
6	4.48 s			4, 13A, 13B, 14, 15	70.20 (d)	4, 5, 7, 8, 10, 11, 15
7					136.90 (s)	
8	4.98 ddt	12.5 (9α), 6.2 (9β), 2.2 (12A, 12B)	9α, 9β	9β, 10	85.37 (d)	
9α	1.69 q	12.5 (8, 9β, 10)	8, 9β, 10	2, 4, 9β	37.52 (t)	1, 8, 10
β	1.78 ddd	12.5 (9α), 6.2 (8), 4.0 (10)	8, 9α, 10	1, 8, 9α, 10		5, 7, 8, 10
10	2.05 dq	12.5 (9α), 4.7(1, 9β)	1, 9α, 9β	1, 8, 9β, 15	37.23 (d)	
11					132.21 (s)	
12A	4.72 dd	14.3 (12B), 2.2 (8)	8, 12B	12B	77.39 (t)	7, 8, 11
B	4.75 dd	14.3 (12A), 2.2 (8)	8, 12A	12A		7, 8, 11
13A	4.18 d	12.2 (13B)	13B	6, 13B, 14	56.36 (t)	7, 11, 12
B	4.25 d	12.2 (13A)	13A	6, 13A, 14		7, 11, 12
14	0.81 d	6.8 (4)	4	3a, 3b, 4, 6, 13A, 3B, 15	16.43 (q)	3, 4, 5
15	0.92 s			1, 3β, 6, 10, 14	17.08 (q)	4, 5, 6, 10

[a] and [b] As in Table 1.

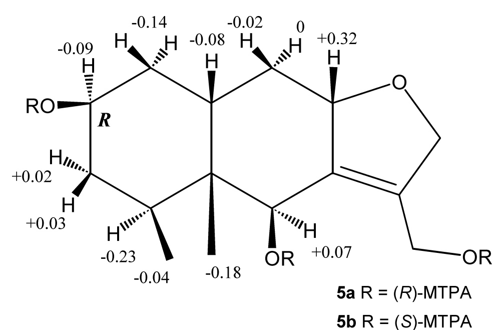

Fig. 5 ^{1}H chemical-shift differences ($\Delta\delta = \delta_S - \delta_R$) between the (*R*)- and (*S*)-MTPA esters (**5a** and **5b**) of peribysin I (**2**).

a modification of the method reported by Miki and co-workers [11]. As shown in Table 4, these compounds inhibited the adhesion of HL-60 cell to HUVEC more potently than herbimycin A [8, 9].

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Table 4 Inhibitory activity of cell adhesion of metabolites

Compound	IC_{50} (μM)
Peribysin H (**1**)	15.2
Peribysin I (**2**)	20.1
Herbimycin A (control)	38.0

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