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Absolute Stereostructures of Cell-adhesion Inhibitors, Peribysins A, E, F and G, Produced by a Sea Hare-derived *Periconia* sp.

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Abstract Peribysins E~G (**1**~**3**) 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. In addition, the absolute configuration of peribysin A (**4**), previously undetermined, has been established by conversion to **2** and **3**. All 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 antitumor materials from microorganisms separated from marine organisms [1~3]. As part of this study, we have previously isolated the cell-adhesion inhibitors, macrosphelides E~I, L [4, 5] and peribysins A (**4**)~D [6], 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 [7, 8]. Further investigation of the metabolites from this fungal strain has now led to the isolation of additional new anti-adhesion compounds designated peribysins E~G (**1**~**3**) (Fig. 1). We describe herein the absolute stereostructures of peribysins E~G (**1**~**3**) and A (**4**) (Fig. 1). The relative configuration of peribysin A has been briefly reported in a preliminary form [6] in addition to their inhibition of cell adhesion.

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 [4~6]. The AcOEt extract of the

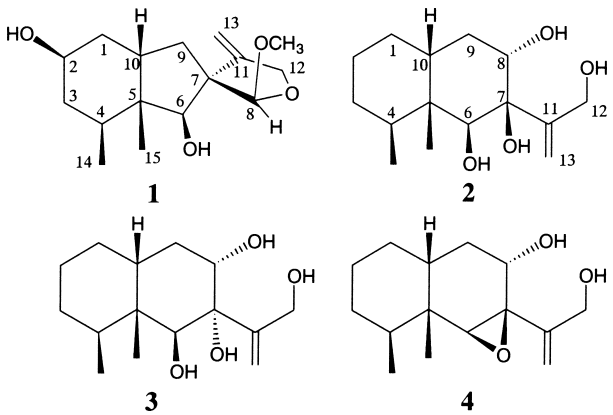

Fig. 1 Structures of peribysins E (**1**), F (**2**), G (**3**) and A (**4**).

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Table 1 Physico-chemical properties of peribysin E∼G (**1**∼**3**)

		1	**2**	**3**
Appearance		Pale yellow oil	Pale yellow oil	Colorless needles (hexane - CH_2Cl_2)
$[\alpha]_D^{22}$		−262.2 (*c* 0.11, EtOH)	−21.5 (*c* 0.10, EtOH)	−1.3 (*c* 0.10, EtOH)
Mp (°C)				187∼189
HREIMS	Found:	282.1827 $(M)^+$	270.1730 $(M)^+$	270.1730 $(M)^+$
	Calcd:	282.1831 (for $C_{16}H_{26}O_4$)	270.1724 (for $C_{15}H_{26}O_4$)	270.1724 (for $C_{15}H_{26}O_4$)
Molecular formula		$C_{16}H_{26}O_4$	$C_{15}H_{26}O_4$	$C_{15}H_{26}O_4$
UV λ_{max} (EtOH) nm (log ε)		208 (3.79), 261 (2.97), 311 (2.64)	205 (3.41)	205 (2.25)
IR ν_{max} (neat) cm^{-1}		3415, 1664	3387, 1639, 1595	3372, 1629, 1593
TLC Rf[a]		0.40	0.16	0.21
Solubility	soluble	DMSO, CH_2Cl_2, MeOH, acetone	DMSO, MeOH, acetone	DMSO, MeOH, acetone
	insoluble	H_2O	CH_2Cl_2, H_2O	CH_2Cl_2, H_2O

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

Table 2 NMR spectral data of peribysin E (**1**) in $CDCl_3$

Position	δ_H[a]	*J*/Hz	^1H-^1H COSY	NOE	δ_C	HMBC (C)[b]
1 α	1.94 dddd	12.8 (1β), 4.8 (2), 2.0 (10), 1.8 (3α)	1β, 2, 10	1β, 2	34.2 (t)	3, 5
β	1.52 ddd	12.8 (1α), 10.8 (2), 5.3 (10)	1α, 2,10	1α, 3β, 10, 15		2, 5, 9
2	3.92 dddd	11.8 (3β), 10.8 (1b), 4.8 (1α), 3.2 (3α)	1α, 1β, 3α, 3β	1α, 3α, 4, 9α	67.1 (d)	
3 α	1.71 dddd	11.8 (3β), 4.0 (4), 3.2 (2), 1.8 (1α)	2, 3β, 4	2, 3β, 4, 14	40.0 (t)	
β	1.27 q	11.8 (2, 3α, 4)	2, 3α, 4	1β, 3α, 14, 15		1, 2, 4
4	1.57 dqd	11.8 (3β), 7.2 (14), 4.0 (3α)	3α, 3β, 14	2, 3α, 6, 9α, 14	35.4 (d)	
5					45.9 (s)	
6	3.56 s			4, 9α, 12α, 13A, 14	88.6 (d)	4, 8, 10, 11
7					60.8 (s)	
8	5.09 s			10, 8-OCH_3	105.5 (d)	6, 11, 12, 8-OCH_3
9 α	1.76 t	13.8 (9β, 10)	9b, 10	1, 4, 6, 9β, 13A	32.9 (t)	1, 5, 7, 8, 11
β	1.89 dd	13.8 (9α), 6.2 (10)	9α, 10	9α, 10		5, 6, 7, 8
10	2.01 m		1α, 1β, 9a, 9β	1β, 8, 9β, 15	45.8 (d)	1, 2, 4, 5
11					152.6 (s)	
12 α	4.49 dt	12.8 (12β), 2.2 (13A, 13B)	12b, 13A, 13B	6, 12β	68.9 (t)	11
β	4.40 dt	12.8 (12α), 2.2 (13A, 13B)	12α, 13A, 13B	12α, 8-OCH_3		
13 A	4.95 t	2.2 (12α, 12b)	12α, 12β	6, 9α, 13B	103.1 (t)	7, 12
B	4.99 t	2.2 (12α, 12β)	12α, 12β	13A		7, 12
14	0.86 d	7.2 (4)	4	3α, 3β, 4, 6, 15	16.1 (q)	3, 4, 5
15	0.93 s			1β, 2β, 10, 14	14.4 (q)	4, 5, 6, 10
6-OH	n.d.					
8-OCH_3	3.38 s			8, 12β	55.1 (q)	8

[a] ^1H 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 ^1H-^{13}C correlations from H to C observed in the HMBC experiment.

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 E∼G (**1**∼**3**). The physico-chemical properties of these compounds are summarized in Table 1.

Peribysin E (**1**) had the molecular formula $C_{16}H_{26}O_4$ established by the $[M]^+$ peak of **1** in HREIMS. The IR spectrum exhibited bands at 3415 and 1664 cm^{-1},

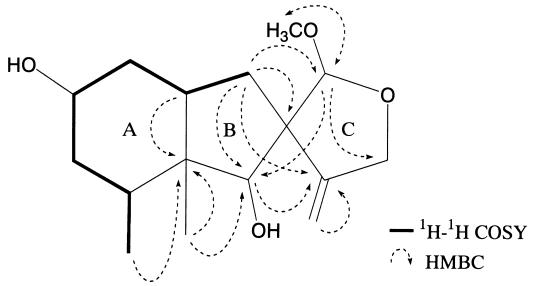

Fig. 2 Selected ^{1}H-^{1}H COSY and HMBC correlations in peribysin E (**1**).

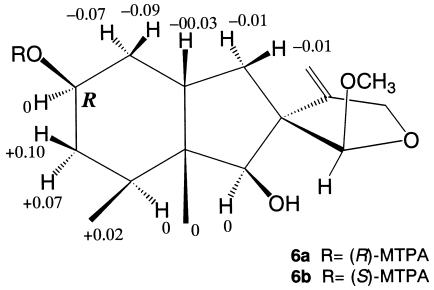

Fig. 4 ^{1}H chemical-shift differences ($\Delta\delta=\delta_S-\delta_R$) between the (*R*)- and (*S*)-MTPA esters **6a** and **6b** of peribysin E (**1**).

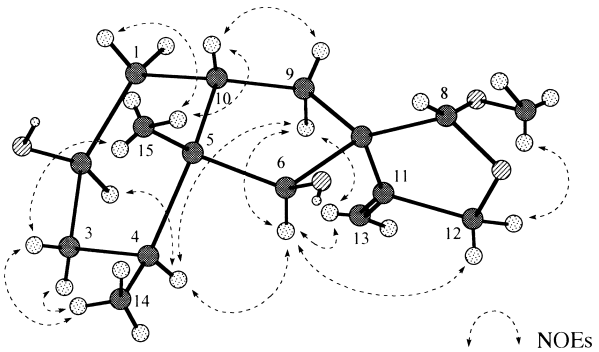

Fig. 3 Observed NOEs for peribysin E (**1**).

characteristic of an alcohol and a double bond. A close inspection of the ^{1}H and ^{13}C NMR spectra of **1** (Table 2) by DEPT and ^{1}H-^{13}C COSY experiments revealed the presence of one vinylidene (C-11 and C-13), one secondary methyl (C-14), one tertiary methyl (C-15), four sp^3-hybridized methylenes (C-1, C-3, C-9 and C-12) including one oxygen-bearing carbon (C-12), 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), two quarternary sp^3-carbons (C-5 and C-7) and one methoxyl group (8-OMe). The ^{1}H-^{1}H 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 diacetate **5**, and protone signals for 2-H and 6-H were downfield-shifted from δ_H 3.92 and δ_H 3.56 to δ_H 5.01 and δ_H 4.87, respectively. This fact implied that the hydroxyl groups exist at C-2 and C-6. Based on the above evidence, the planar structure of **1** was elucidated.

The stereochemistry of **1** was deduced from NOESY experiments (Table 2, 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 from 15-H to 10-H, and from 14-H to 3β-H suggested that 5-methyl group is oriented *cis* to both 10-H and 4-methyl group in equatorial arrangements. In addition to NOEs from 6-H to 4-H, 9α-H and 13A-H, NOE between 10-H and 9β-H was implied that 6-H is oriented *cis* to both 4-H and C-7–C-11 bond, and therefore C-7–C-8 bond is oriented *cis* to both 10-H and 9β-H. Furthermore, NOE correlations (12β-H/8-OMe and 12α-H/6-H) showed that 8-H is *cis* to C-7–C-6 bond. Based on the evidence summarized above, the relative stereostructure **1** for peribysin E was elucidated as depicted in Fig. 3.

The modified Mosher method [9] was applied to determine the absolute configuration of peribysin E (**1**). The ^{1}H chemical-shift differences between the (*R*)- and (*S*)-MTPA esters **6a** and **6b** of compound **1** are shown in Fig. 4. The result suggested that a 2*R* configuration, and hence allowed assignment of absolute stereostructure **1** as 2*R*, 4*S*, 5*R*, 6*S*, 7*S*, 8*R*, 10*R* configuration for peribysin E.

Peribysin F (**2**) had the molecular formula $C_{15}H_{26}O_4$ established by HREIMS. Close inspection of the ^{1}H and ^{13}C NMR spectra of **2** (Table 3) by DEPT and ^{1}H-^{13}C COSY experiments revealed the presence of one vinylidene (C-11 and C-13), one secondary methyl (C-14), one tertiary methyl (C-15), five sp^3-hybridized methylenes (C-1, C-2, C-3, C-9 and C-12) including one hydroxymethyl (C-12), four sp^3-methines (C-4, C-6, C-8 and C-10) including two oxymethines (C-6 and C-8), two sp^3 quaternary carbons (C-5 and C-7) including one oxygen-bearing carbon (C-7). Analysis of The ^{1}H-^{1}H COSY and HMBC (from H-6 to C-5, C-7, C-8, C-10 and C-11, from H-13 to C-7 and C-12, and from H-12B to C-7, C-11 and C-13) correlations led to the planar structure for **2**. Furthermore, acetylation of **2** gave the triacetate **7**, and proton signals for 6-H, 8-H and 12-H were downfield-shifted to δ_H 5.62, δ_H 5.14 and δ_H 4.72, respectively. This fact implied that the tertiary hydroxyl groups exist at C-6, C-8 and C-12. In addition the

Table 3 NMR spectral data of peribysins F (**2**) and G (**3**) in MeOH-d_4

Position	**2**		**3**	
	δ_H[a]	δ_C	δ_H[a]	δ_C
1	1.62 m	29.3 (t)	α 1.32 dt β 1.77 tt	28.5 (t)
2	1.44 m	22.0 (t)	α 1.58 m β 1.44 dtt	22.2 (t)
3	α 1.48 m β 1.28 m	31.7 (t)	1.32 m	32.2 (t)
4	2.05 m	33.3 (d)	2.80 dqd	30.7 (d)
5		42.5 (s)		42.1 (s)
6	4.01 s	74.7 (d)	3.60 s	79.0 (d)
7		80.5 (s)		80.9 (s)
8	3.87 dd	76.2 (d)	4.17 dd	70.2 (d)
9	α 1.97 m β 1.77 br d	33.8 (t)	2.25 q 1.32 m	32.5 (t)
10	1.90 m	38.3 (d)	1.80 dq	37.6 (d)
11		151.7 (s)		154.7 (s)
12	A 4.22 d B 4.31 d	64.7 (t)	4.29 s	65.1 (t)
13	A 5.43 s B 5.63 s	116.8 (t)	5.37 s 5.45 s	115.8 (t)
14	0.83 d	17.1 (q)	0.81 d	17.3 (q)
15	1.04 s	18.5 (q)	0.92 s	18.1 (q)

[a] As in Table 1.

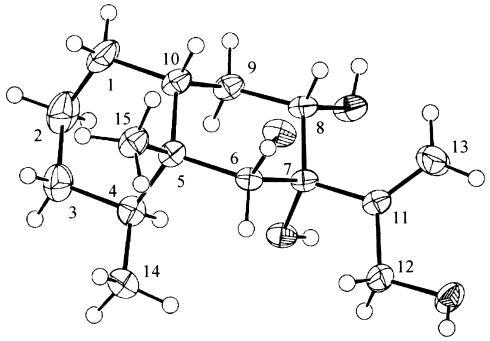

Fig. 5 X-Ray crystal structure for peribysin G (**3**).

presence of a hydroxy group at the remaining oxygenated carbon (C-7) was deduced from its carbon chemical-shift (δ_C 80.53) and the molecular formula of **2**. In NOESY experiment, NOEs (15-H to 1β-H, 3β-H, 10-H and 14-H) observed in A ring of **2** were the same as those of peribysin E (**1**), implying that the A ring exists in a chair conformation . In addition, NOEs (10-H to 8-H, 9α-H to 4-H, 6-H to 4-H and 121-H, and 13B-H to 4-H) implied that the B ring exists in a chair conformation with the 8-H, 10-H and 6-hydroxy group in coaxial arrangements and with 5-methyl group and 7-hydroxy group in equatorial arrangements. Based on the above evidence, the relative stereostructure **2** for peribysin F was elucidated. The absolute configuration of **2** is described later.

Peribysin G (**3**) had the same molecular formula as **2** based on HREIMS. The general features of the ^{1}H and ^{13}C NMR spectra (Table 3) closely resembled those of **2** except that the proton signal for 4-H (δ_H 2.05) and 6-H (δ_H 4.01), and the carbon signal for C-6 (δ_C 74.71) and C-8 (δ_C 76.15) in **3** revealed a chemical shift difference relative to those of **2**. The ^{1}H-^{1}H COSY and HMBC (from 6-H to C-5, C-7, C-8, C-10 and C-11, from 13-H to C-7 and C-12, and from 12-H to C-7, C-11 and C-13) correlations (Table 3) implied that peribysin G (**3**) has the same planar structure as **2**. In NOESY experiment, NOEs (15-H to 1β-H, 3-H, 10-H and 14-H) observed in A ring of **3** were the same as those of above peribysins, implying that the A ring exists in a twist-chair conformation with 1β-H, 3β-H and 5-methyl group in coaxial 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 chair conformation with the 8-H, 10-H and 6-hydroxy group in coaxial arrangements and with 5-methyl group in equatorial arrangements. In addition, an X-ray crystal-structure analysis was carried out for a single crystal of **3** (obtained by recrystallization from hexane-CH_2Cl_2) (Fig. 5). Based on the above evidence, peribysin G (**3**) was found to be the C-7 stereoisomer of peribysin F (**2**). The absolute configuration of **3** was elucidated by application of CD spectrum [10]. Bis-*p*-bromobenzoate **8** derived from peribysin G (**3**) exhibited positive first and negative second Cotton effect [253 nm ($\Delta\varepsilon$ +8.8) and 238 nm ($\Delta\varepsilon$ −4.5)] in the CD spectrum. This evidence allowed assignment of the absolute configuration of **3** as shown in Fig. 5.

The relative stereostructure of peribysin A (**4**) has already been reported previously [6], but its absolute configuration has remained undecided. The modified Mosher method was not applied for determination of the absolute configuration of **4**, because the 8-OH, the secondary hydroxy group in **4**, had not been derivatized to an MTPA ester. Therefore peribysin A (**4**) was converted to peribysin G (**3**), the absolute stereostructure of which had already been determined. The acid-catalyzed hydrosis of **4** gave both peribysin G (**3**) and F (**2**), however peribysin F (**2**) and G (**3**) were not artefacts because of the potent stability of **4** in various solvent. Based on this evidence, the absolute configuration of peribysin A (**4**) and F (**2**) were elucidated.

Table 4 Inhibitory activities against cell adhesion of metabolites

Compound		IC_{50} (μM)
Peribysin	E (**1**)	11.5
	F (**2**)	20.7
	G (**3**)	15.6
Herbimycin A	(standard)	38.0

Peribysins E～G (**1**～**3**) were examined using herbimycin A [7, 8] as a standard sample in the adhesion assay system using HL-60 cells and HUVEC, according to a modification of the method reported by Miki and co-workers [11]. As shown in Table 4, all of these compounds inhibited the adhesion of HL-60 cell to HUVEC more potently than herbimycin A.

Experimental

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. ORD and CD spectra 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 aluminum sheets (DC-Alufolien Kieselgel 60 F_{254}, 0.2 mm) with the solvent system CH_2Cl_2 - MeOH (19 : 1), and compounds were 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%, glucose 1% 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 (2 : 98) eluate (172.6 mg) from silica gel column chromatography was purified by HPLC using MeOH - H_2O (7 : 3) as the eluent to afford **1** (17.8 mg, 0.31%). The MeOH - CH_2Cl_2 (5 : 95) eluate (223.8 mg) from silica gel column chromatography was purified by HPLC using MeOH - H_2O (6 : 4) as the eluent to afford **2** (12.7 mg, 0.22%), **3** (8.8 mg, 0.15%).

Acetylation of Peribysin E (1)

To a solution of **1** (2.2 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 diacetate **5** (1.8 mg) as a pale yellow oil.

Diacetate **5**: $[\alpha]_D$ +35.0 (*c* 0.069, EtOH); IR ν_{max} (neat) cm^{-1} 1738 (ester), 1602, 1586 (C=C); EIMS *m/z* 366 (M^+, 1.0%); HREIMS *m/z* for $C_{20}H_{30}O_6$ (M^+), Calcd: 366.0966; Found: 366.0948; ^1H NMR δ ppm ($CDCl_3$): 0.84 (3H, d, *J*=7.2 Hz, 14-H), 0.86 (3H, s, 15-H), 1.29 (1H, q, *J*=11.8 Hz, 3β-H), 1.63 (1H, ddd, *J*=12.7, 10.8, 5.1 Hz, 1β-H), 1.75 (1H, dqd, *J*=11.8, 7.2, 3.8 Hz, 4-H), 1.80 (1H, dddd, *J*=11.8, 3.8, 3.0, 1.9 Hz, 3α-H), 1.89 (1H, t, 13.5, 9α-H), 1.94 (1H, dddd, *J*=12.7, 4.8, 2.0, 1.9 Hz, 1α-H), 2.03 (3H, s, COOC*H*$_3$), 2.04 (1H, m, 9β-H), 2.06 (1H, m, 10-H), 2.07 (3H, s, COOC*H*$_3$), 3.35 (3H, s, 8-OC*H*$_3$), 4.28 (1H, dt, *J*=12.8, 2.3 Hz, 12β-H), 4.39 (1H, dt, *J*=12.8, 2.3 Hz, 12α-H), 4.87 (1H, s, 6-H), 4.93 (1H, s, 8-H), 5.00 (1H, t, *J*=2.3 Hz, 13A-H), 5.01 (1H, m, 2-H), 5.03 (1H, t, *J*=2.3 Hz, 13B-H).

Formation of the (*R*)- and (*S*)-MTPA Esters 6a and 6b from Peribysin E (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 E (**1**) (0.6 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 **6a** (0.7 mg) as an amorphous powder. The same reaction with **1** (0.7 mg) using (*S*)-MTPA (2.2 mg) gave ester **6b** (0.8 mg).

Ester **6a**: EIMS *m/z* 498 (M^+, 3.0%); HREIMS *m/z* for $C_{26}H_{33}F_3O_6$ (M^+), Calcd: 498.2228; Found: 498.2224; ^1H NMR δ ppm ($CDCl_3$): 0.86 (3H, d, *J*=6.9 Hz, 14-H), 0.92 (3H, s, 15-H), 1.37 (1H, q, *J*=12.6 Hz, 3β-H), 1.65 (1H, m, 4-H), 1.76 (1H, m, 1β-H), 1.78 (1H, m, 3α-H), 1.81 (1H, t, *J*=13.5 Hz, 9α-H), 1.95 (1H, dd, *J*=13.5, 5.5 Hz, 9β-H), 2.01 (1H, m, 1α-H), 2.03 (1H, m, 10-H), 2.17 (1H, br s, 6-OH), 3.38 (3H, s, 8-OMe), 3.56 (3H, s, OMe), 3.57 (1H, s, 6-H), 4.41 (1H, dt, *J*=12.8, 2.2 Hz, 12β-H), 4.49 (1H, dt,

J=12.8, 2.2 Hz, 12α-H), 4.99 (1H, t, J=2.2 Hz, 13A-H), 5.01 (1H, t, J=2.2 Hz, 13B-H), 5.06 (1H, s, 8-H), 5.27 (1H, dddd, J=12.5, 10.5, 5.0, 3.1 Hz, 2-H), 7.41 (3H, m, Ar.H) and 7.53 (2H, m, Ar.H).

Ester **6b**: EIMS m/z 498 (M^+, 2.2%); HREIMS m/z for $C_{26}H_{33}F_3O_6$ (M^+), Calcd: 498.2228; Found: 498.2224; ^{1}H NMR δ ppm ($CDCl_3$): 0.88 (3H, d, J=6.9 Hz, 14-H), 0.92 (3H, s, 15-H), 1.47 (1H, q, J=12.4 Hz, 3β-H), 1.65 (1H, m, 4-H), 1.67 (1H, m, 1β-H), 1.80 (1H, t, J=13.5 Hz, 9α-H), 1.85 (1H, m, 3α-H), 1.94 (1H, m, 1α-H), 1.94 (1H, m, 9β-H), 2.00 (1H, m, 10-H), 2.17 (1H, br s, 6-OH), 3.37 (3H, s, 8-OMe), 3.57 (3H, s, OMe), 3.57 (1H, s, 6-H), 4.41 (1H, dt, J=12.8, 2.2 Hz, 12β-H), 4.49 (1H, dt, J=12.8, 2.2 Hz, 12α-H), 4.98 (1H, t, J=2.2 Hz, 13A-H), 5.01 (1H, t, J=2.2 Hz, 13B-H), 5.06 (1H, s, 8-H), 5.27 (1H, dddd, J=12.4, 10.6, 5.0, 3.2 Hz, 2-H), 7.41 (3H, m, Ar.H) and 7.53 (2H, m, Ar.H).

Acetylation of Peribysin F (4)

Using the same procedure as above with compound **1**, a solution of **4** (1.5 mg) in pyridine (1.0 ml) was treated with Ac_2O (1.0 ml) and purified by HPLC [MeOH - H_2O (9 : 1)] to afford triacetate **7** (1.3 mg) as a pale yellow oil.

Triacetate **7**: $[\alpha]_D$ +9.8 (c 0.079, EtOH); IR ν_{max} (neat) cm^{-1} 3485 (OH), 1743 (ester), 1653, 1595 (C=C); EIMS m/z 396 (M^+, 0.3%); HREIMS m/z for $C_{21}H_{32}O_7$ (M^+), Calcd: 396.1677; Found: 396.1658; ^{1}H NMR δ ppm ($CDCl_3$): 0.86 (3H, d, J=7.1 Hz, 14-H), 1.04 (3H, s, 15-H), 1.28 (1H, m, 3β-H), 1.43 (2H, m, 2-H), 1.44 (1H, m, 3α-H), 1.58 (2H, m, 9-H), 1.58 (2H, m, 1-H), 1.74 (1H, br s, 4-H), 1.92 (1H, br s, 10-H), 2.02 (3H, s, COOCH_3), 2.08 (3H, s, COOCH_3), 2.08 (3H, s, COOCH_3), 2.80 (1H, s, 7-OH), 4.72 (2H, s, 12-H), 5.14 (H, br s, 8-H), 5.37 (1H, s, 13A-H), 5.58 (1H, s, 13B-H), 5.62 (1H, s, 6-H).

X-Ray Crystallography of Peribysin G (3)

Peribysin G (**3**) was crystallized from hexane - CH_2Cl_2 solution by the vapor diffusion method. Crystal data: $C_{15}H_{26}O_4$, M_r=270.36, monoclinic, $P2_1$, a=7.960 (2) Å, b=8.605 (2) Å, c=10.955 (3) Å, b=106.855 (4)°, V=718.1 (3) Å^3, Z=2, F(000)=296, μ(Mo-Kα)=0.089 mm^{-1}. Data collection was performed by a Bruker smart APEX using Mo-Kα radiation. Total 6292 reflections were collected until θ=27.10 Å, in which 1685 reflections were observed (I>2σ(I)). The crystal structure was solved by the direct method using SHELXS-97 [12]. The structure was refined by the full matrix least-squares method on F using SHELXL-97 [12]. In the structure refinements, non-hydrogen atoms were refined with anisotropic temperature factors. Hydrogen atoms were calculated on the geometrically ideal positions by the 'ride on' method, and were included in the calculation of structure factors with isotropic temperature factors. In the final stage, R=0.0440, wR=0.1224 and S=1.198 were obtained.

Formation of Bis-*p*-bromobenzoate 8 from Peribysin G (3)

To a solution of **3** (0.78 mg) in pyridine (0.1 ml) was added *p*-bromobenzoylchloride (1.6 mg), and the reaction mixture was left at room temperature for 1 hour. 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 **8** (0.69 mg) as-pale yellow oil.

Bis-*p*-bromobenzoate **8**: EIMS m/z 636 (M^+, 6.7%); HREIMS m/z for $C_{29}H_{32}Br_2O_6$ (M^+), Calcd: 636.0536; Found: 636.0533; ^{1}H NMR δ ppm ($CDCl_3$): 0.86 (3H, d, J=6.6 Hz, 14-H), 1.05 (3H, s, 15-H), 1.26 (1H, m, 3β-H), 1.34 (1H, m, 3α-H), 1.37 (1H, m, 1α-H), 1.49 (2H, m, 2-H), 1.65 (1H, ddd, J=12.8, 4.8, 3.9 Hz, 9β-H), 1.75 (1H, m, 1β-H), 2.07 (1H, m, 10-H), 2.30 (1H, d, J=3.2 Hz, 6-OH), 2.40 (1H, q, J=12.8 Hz, 9α-H), 2.77 (1H, d, J=3.2 Hz, 6-H), 4.93 (1H, d, J=13.3 Hz, 12A-H), 5.01 (1H, d, J=13.3 Hz, 12B-H), 5.36 (1H, s, 13A-H), 5.37 (1H, s, 13B-H), 5.72 (1H, dd, J=12.8, 4.8 Hz, 8-H), 7.54 (2H, d, J=8.8 Hz, Ar.H), 7.56 (2H, d, J=8.8 Hz, Ar.H), 7.79 (2H, d, J=8.8 Hz, Ar.H), 7.84 (2H, d, J=8.8 Hz, Ar.H).

Formation of Peribysin F (2) and G (3) from Peribysin A (4)

To a solution of **4** (6.7 mg) in MeOH (0.1 ml) was added H_2O (2.0 ml) and c-HCl (1 drop), and the reaction mixture was left at room temperature for 1 hour. The mixture was concentrated to dryness under reduced pressure, and residue was purified by HPLC using MeOH - H_2O (6 : 4) as the eluent to afford **2** (1.77 mg) and **3** (2.56 mg), the spectral date of which were identical with the natural products.

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 [9]. 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 incubated 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).

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