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Peribysins A–D, potent cell-adhesion inhibitors from a sea hare-derived culture of *Periconia* species

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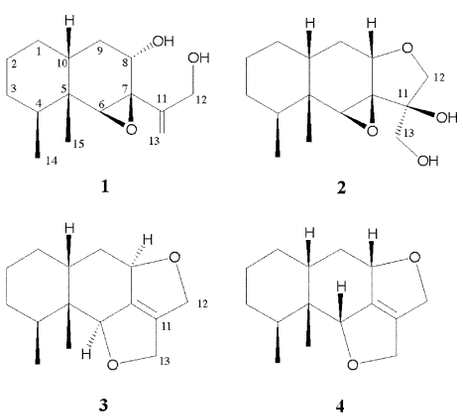
Received 26th March 2004, Accepted 1st June 2004
First published as an Advance Article on the web 30th June 2004

Peribysins A–D **1**–**4**, including a new type of furanofuran, have been isolated from a strain of *Periconia byssoides* originally separated from the sea hare *Aplysia kurodai*, and their relative stereostructures have been elucidated on the basis of NMR spectral analyses. All these metabolites potently inhibited the adhesion of human-leukemia HL-60 cells to HUVEC. The activity of compound **4**, exhibiting the most potent inhibitory activity, was 380 times as potent as herbimycin A (standard).

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 separated from marine organisms.[1–3] As part of this study, we have previously isolated pericosines A and B,[4] and macrosphelides E–I and L, the six 16-membered macrolides,[5,6] from a strain of *Periconia byssoides* OUPS-N133 originally separated from the sea hare *Aplysia kurodai*. Pericosine A showed significant *in vivo* tumor-inhibitory activity, while macrosphelides E–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 metabolites of this fungal strain has now led to the isolation of additional new anti-adhesion compounds, designated peribysins A–D **1**–**4**, which include a new type of furanofuran. Their inhibition of cell adhesion was more potent than that of macrosphelides. Cell-adhesion inhibitors are expected as useful leads for the control of cancer metastasis. Isolation of macrosphelides with inhibition of cell adhesion from a strain of *P. byssoides* has led us to examine metabolites of this fungal strain further. In the separation, four new eremophilane sesquiterpenoids including a new type of furanofuran were isolated as anti-adhesion compounds, and designated peribysins A–D **1**–**4**. Their inhibitory activities of cell adhesion are more potent than that of the strongest inhibitor, macrosphelide H, of macrosphelides reported previously.[5] We describe herein the determination of their chemical structures, and their inhibition of cell adhesion.



1 **2**

3 **4**

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 culture filtrate was purified by fractionation employing a combination of Sephadex LH-20 and silica gel column chromatographies and HPLC to afford peribysins A–D **1**–**4**.

Peribysin A **1** has the molecular formula $C_{15}H_{24}O_3$ established by the $[M + H]^+$ peak of **1** in high-resolution electron-impact mass spectrometry (HREIMS). Its IR spectrum exhibited bands at 3347 and 1651 cm^{-1}, characteristic of an alcohol and a double bond. Close inspection of the ^1H and ^{13}C NMR spectra of **1** (Table 1) by DEPT and ^1H–^{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, C9and 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) and two hydroxy groups. The ^1H–^1H COSY analysis of **1** led to two partial structural units as shown by bold-faced lines in Fig. 1, which were supported by HMBC correlations (Table 1). The connection of these three units and the remaining functional groups was determined on the basis of the key HMBC correlations summarized in Fig. 1, and the planar structure of **1** was elucidated. The presence of an epoxide between C-6 and C-7 was supported by the large J_{C-H} value (178 Hz) of C-6 characteristic for three-membered rings.[9]

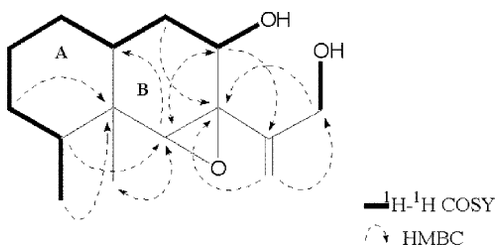


Fig. 1 Slected ^1H–^1H COPSY and HMBC correlations in peribysin A **1**.

The stereochemistry of **1** was deduced from NOESY experiments (Table 1, Fig. 2). NOE correlations from H-15 to H-1β and H-3β implied that the A ring exists in a chair conformation

DOI: 10.1039/b404459b

Table 1 NMR spectral data of peribysin A **1** in $CDCl_3$

Position	δ_H [a]		*J*/Hz	^1H–^1H COSY	NOE	δ_C	HMBC (C) [c]
1α	1.33	br d	11.0 (1β)	2, 10	2, 10	26.89 (s) [b]	2, 3, 5
1β	1.68	m		2, 10	7, 3β, 10, 15		2, 3, 9, 10
2	1.44	m		1α, 1β, 3α, 3β	1α, 1β, 3α, 3β, 4	20.44 (s)	1, 3, 4, 10
3α	1.46	m		2, 4	2, 4, 14	30.71 (s)	1, 2, 4, 5, 14
3β	1.26	dtd	14.3 (3α), 11.2 (4, 2α), 2.8 (2β)	2, 4	1β, 2, 14, 15		2, 4, 14
4	1.95	dqd	11.2 (3β), 6.4 (14), 3.8 (3α)	3α, 3β, 14	2, 3α, 6, 14	31.99 (t)	2, 3, 5, 6, 9, 14, 15
5						35.73 (q)	
6	3.18	s			4, 13A, 14, 15	69.90 (t)	5, 7, 8, 10, 11, 15
7						67.51 (q)	
8	3.98	dd	10.9 (9α), 5.8 (9β)	9α, 8-OH	9β, 10	68.40 (t)	6, 9, 10, 11
9α	1.81	dt	14.3 (9β), 10.9 (8, 10)	8, 10		32.46 (s)	1, 5, 7, 8, 10
9β	1.50	m		10	8, 10		1, 5, 7, 8, 10
10	1.52	m		1α, 1β, 9α, 9β	1α, 1β, 8, 9β, 15	33.12 (t)	1, 2, 4, 9, 15
11							
12A	4.12	d	11.3 (12B)	12B	12B, 13B	63.71 (s)	7, 11, 13
12B	4.28	d	11.3 (12A)	12A, 12-OH	12A		7, 11, 13
13A	5.19	s			6	117.34 (s)	7, 11, 12
13B	5.31	s			12A		7, 11, 12
14	0.95	d	6.4 (4)	4	3α, 3β, 4, 6, 15	16.64 (p)	3, 4, 5
15	1.05	s			1β, 3β, 6, 10, 14	16.64 (p)	4, 5, 6, 10
8-OH	4.23	br s		8			
12-OH	4.23	br s		12B			

[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] Letters, p, s, t and q, in parentheses indicate respectively primary, secondary, tertiary and quaternary carbons, assigned by DEPT. [c] Long range ^1H–^{13}C corrections from H to C observed in the HMBC experiment.

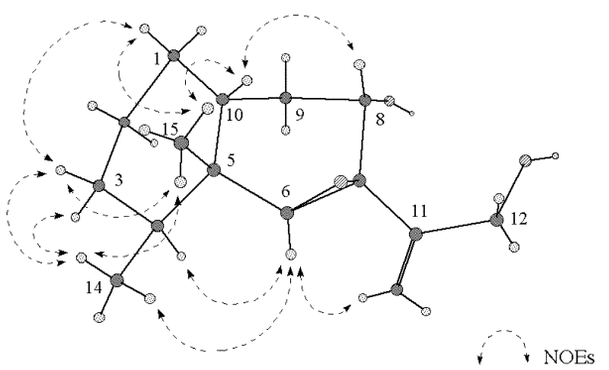


Fig. 2 Observed NOEs for peribysin A **1** (graphical reprensentation using the program Chem 3D).

with the 5-methyl group, H-1β and H-3β in coaxial arrangements. NOEs from H-15 to H-10 and H-14 suggested that the 5-methyl group is oriented *cis* to both H-10 and the 4-methyl group in equatorial arrangements. In addition to NOEs from H-6 to H-15, H-14 and H-3, NOE between H-8 and H-10 implied that the B ring exists in a twist-chair conformation with H-8 and H-10 in a coaxial arrangement and with H-6 in an equatorial arrangement (*trans* to the 5-methyl group). This evidence led to relative stereostructure **1** for peribysin A.

Peribysin B **2** was assigned a molecular formula which contained one oxygen atom more than that of **1** as deduced from HREIMS. The general features of its ^1H and ^{13}C NMR spectra closely resembled those of **1** except that the signals for the vinylidene group (C-11 and C-13) in **1** were replaced by those of an sp^3 quaternary carbon [δ_C 77.60 (C-11)] and an sp^3 methylene [δ_H 3.54, 3.77 (H-13); δ_C 64.19 (C-13)] each bearing an oxygen in **2** (Table 2). Observation of an HMBC correlation between H-8 and C-12 implied that the ether linkage is between C-3 and C-12. This fact suggested that C-11 is linked to a hydroxy group because of the presence of the two hydroxy groups in **2**. Other HMBC correlations (H-6/C-11, H-8/C-11, H-12/C-7, H-13/C-7, and H-13/C-12) led to the planar structure of **2** with a five-membered ether ring (Table 2). Since NOEs observed in the A and B rings of **2** are the same as those of **1**, the stereochemistry of **2** except for C-11 is the same as that of **1**. Observation of NOE between H-1 and H-13 showed the C-11–C-13 bond to be *cis* to H-6 (Table 2). Based on this evidence, the relative stereostructure **2** for peribysin B was elucidated.

Peribysin C **3** was assigned the molecular formula $C_{15}H_{22}O_2$ as deduced from HREIMS. The general features of its ^1H and ^{13}C NMR spectra closely resembled those of **2** except that the signals for two hydroxy groups disappeared from **3** and the signals for H-6, H-8, H-13, C-5–C-10 and C-11–C-13 in **3** revealed a chemical shift difference relative to those of **2**, suggesting the presence of a tetrasubstituted ethylene and two ether linkages in **3** (Table 3). Analysis of HMBC correlations (H-13B to C-6, C-7, C-11 and C-12, and H-12B to C-7, C-8, C-11 and C-13) led to the planar structure for **3** (Table 3). NOEs (H-15 to H-1β, H-3β, H-10 and H-14) observed in the A ring of **3** were the same as those of **1**, implying that the A ring exists in a chair conformation with the 5-methyl group, H-1β and H-3β in coaxial arrangements and with H-10 and the 4-methyl group in equatorial arrangements. Furthermore, NOEs from H-6 to H-4 and H-14, from H-9α to H-4 and H-8, and from H-9β to H-10 implied that the B ring exists in a twist-boat conformation with H-8, H-9α, H-4 and H-6 on the same side and on the opposite side to H-10 and the 5-methyl group (Table 3). This evidence led to relative stereostructure **3** for peribysin C, belonging to a new type of furanofuran.

Peribysin D **4** had the same molecular formula as **3** as deduced from HREIMS. The general features of its ^1H and ^{13}C NMR spectra closely resembled those of **3** except that the signals for C-1, C-4, C-6, C-8, C-10, C-12 and C-13 revealed a chemical shift difference relative to those of **3** (Table 4). Analysis of ^1H–^1H COSY and HMBC correlations suggested that **4** is the stereoisomer of **3** (Table 4). NOEs from H-14 to H-2β, H-10, H-3β and H-15 implied that the A ring of **4** exists in a chair conformation with the 4-methyl group, H-10 and H-2β in coaxial arrangements and with the 5-methyl group in an equatorial arrangement (Table 4, Fig. 3). NOEs from H-15 to H-6, H-9β and H-10, and from H-9β to H-8 indicated that the B ring exists in a twist-boat conformation with the 5-methyl group, H-6, H-8, and H-9β on the same side. The above evidence led to relative stereostructure **4** for peribysin D. Work on the absolute configurations of these new compounds is in progress.

Peribysins A–D **1**–**4** 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

Table 2 NMR spectral data of peribysin B **2** in $CDCl_3$

Position	δ_H [a]		*J*/Hz	^1H–^1H COSY	NOE	δ_C	HMBC (C) [c]
1α	1.36	m		2	2, 10	27.04 (s) [b]	3, 5, 9, 10
1β	1.70	tt	13.1 (1α, 2α), 4.2 (2β, 10)	2, 10	2, 8β, 10, 15		3, 5, 9, 10
2	1.46	m		1α, 1β, 3β	1α, 1β, 3α, 3β, 4	20.55 (s)	1, 3, 4, 10
3α	1.47	m		4	2, 4, 14	30.24 (s)	2, 4, 5, 14
3β	1.26	dtd	13.1 (3α), 10.8 (2α, 4), 4.2 (2β)	2, 4	1β, 2, 14, 15		1, 2, 4, 5, 14
4	1.85	dqd	11.4 (3β), 6.8 (14), 3.5 (3α)	3α, 3β, 14	2, 3α, 6, 14	30.77 (t)	2, 3, 6, 14, 15
5						35.83 (q)	
6	3.33	s			4, 13A, 13B, 14	65.02 (t)	4, 7, 8, 10, 11, 15
7						72.62 (q)	
8	3.84	dd	10.1 (9α), 7.1 (9β)	9α	9β, 10	70.56 (t)	6, 9, 10, 11, 12
9α	1.87	m		8, 10		30.86 (s)	1, 5, 7, 8, 10
9β	1.52	ddd	12.3 (9α), 7.1 (8), 2.2 (10)	8, 10	8, 10		1, 5, 7, 10
10	1.34	m		1β, 9α, 9β	1α, 1β, 8, 9β, 15	32.50 (t)	1, 2, 4, 5, 6, 8, 9, 15
11						77.60 (q)	
12α	3.86	d	10.2 (12β)	12β	13A, 13B	76.43 (s)	7, 8, 11, 13
12β	3.98	d	10.2 (12α)	12α			7, 8, 11
13A	3.54	d	11.8 (13B)	13B	6, 12α	64.19 (s)	7, 12
13B	3.77	d	11.8 (13A)	13A, 13-OH	6, 12α		7, 11, 12
14	0.96	d	6.8 (4)	4	3α, 3β, 4, 6, 15	16.93 (p)	3, 4, 5
15	1.07	s			1β, 3β, 10, 14	16.52 (p)	4, 5, 6, 10
11-OH	2.55	br s					
13-OH	2.28	br s		13B			

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

Table 3 NMR spectral data of peribysin C **3** in $CDCl_3$

Position	δ_H [a]	*J*/Hz	^1H–^1H COPY	NOE	δ_C	HMBC (C) [c]
1α	1.32 m		2α, 2β, 10	2α, 2β, 9β, 10	26.37 (s) [b]	3, 5, 9, 10
1β	1.72 m		2α, 2β, 10	2β, 3β, 10, 15		3, 5, 9, 10
2α	1.44 m		1α, 1β, 3α, 3β	1α, 3α, 4	20.17 (s)	1, 3, 4, 10
2β	1.52 d qnt	14.8 (2α), 4.5 (1α, 1β, 3α, 3β)	1α, 1β, 3α	1α, 1β, 3α, 3β		1, 3, 4, 10
3α	1.40 m		2α, 2β	2α, 2β, 4, 14	30.80 (s)	1, 4, 5, 14
3β	1.29 dtd	12.8 (3α), 10.5 (2α, 4), 4.2 (2β)	2α, 2β, 4	1β, 2α, 14, 15		1, 2, 4, 5, 14
4	1.60 dqd	10.5 (3β), 6.9 (14), 1.8 (3α)	3β, 14	2α, 3α, 6, 9α, 14	29.46 (t)	3, 5, 14, 15
5					41.48 (q)	
6	4.53 s			4, 14	69.98 (t)	4, 5, 7, 8, 10, 11, 15
7					136.72 (q)	
8	5.06 dddd	8.9 (9α), 5.3 (12A), 3.0 (12B), 0.8 (9β)	9α, 9β	9α, 9β	84.12 (t)	7, 9, 11
9α	1.90 ddd	13.8 (9β), 10.4 (10), 8.9 (8)	8, 10	4, 8	35.23 (s)	1, 5, 7, 8, 10
9β	1.73 ddd	13.8 (9α), 1.4 (10), 0.8 (8)	8, 10	1α, 8, 10,		1, 5, 7, 8
10	1.94 m		1α, 1β, 9α, 9β	1α, 1β, 9β, 15	34.52 (t)	2, 4, 8, 9, 15
11					131.02 (q)	
12A	4.78 dd	12.3 (12B), 5.3 (8)	12B	12B	76.37 (s)	7, 8, 11
12B	4.81 dd	12.3 (12A), 3.0 (8)	12A	12A		7, 8, 11, 13
13A	4.15 d	13.0 (13B)	13B	13B	56.03 (s)	7, 11, 12
13B	4.35 d	13.0 (13A)	13A	13A		6, 7, 11, 12
14	0.73 d	6.9 (4)	4	3α, 3β, 4, 6, 15	16.00 (p)	3, 4, 5
15	1.04 s			1β, 3β, 10, 14	16.38 (p)	4, 5, 6, 10

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

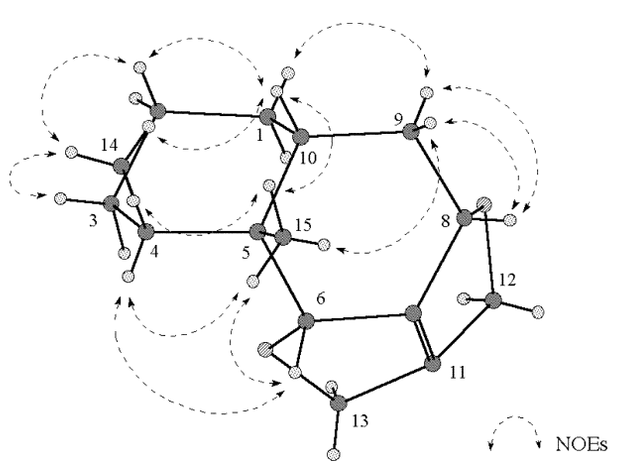


Fig. 3 Observed NOEs for peribysin D **4** (graphical representation using the programme Chem 3D).

reported by Miki and co-workers.[10] As shown in Table 5, all of these compounds inhibited the adhesion of HL-60 cells to HUVEC more potently than herbimycin A. Among them, **1** and **4** exhibited especially potent inhibitory activities, and their activities were 190 to 380 times as potent as herbimycin A, and 30 to 86 times as potent as the strongest inhibitor, macrosphelide H, of macrosphelides reported previously.[5] The inhibitory activity of **4** is more potent than that of its stereoisomer **3**, implying that the stereochemistry plays an important role in the activity.

Experimental

General procedures

UV spectra were recorded on a Shimadzu spectrophotometer and IR spectra on a Perkin Elmer FT-IR spectrometer 1720X. 1D and 2D NMR spectra were recorded at 27 °C on a Varian UNITY INOVA-500 spectrometer, operating at 500 and 125.7

Table 4 NMR spectral data of peribysin D **4** in $CDCl_3$

Position	δ_H [a]	*J*/Hz	^1H–^1H COSY	NOE	δ_C	HMBC (C) [c]
1α	1.92 m		2α, 2β	2α, 3α	30.20 (s) [b]	3, 5, 9, 10
1β	1.46 m		2α, 2β, 10	2α, 2β, 9α, 10		3, 5
2α	1.51 m		1α, 3α, 3β	1α, 1β, 3α, 3β	20.98 (s)	1, 4, 10
2β	1.48 m		1α, 1β, 3α, 3β	1β, 3β, 10, 14		3, 4, 10
3α	1.25 m		2α, 2β, 4	1α, 2α, 4	28.89 (s)	1, 2, 4, 5
3β	1.35 m		2β, 4	2α, 2β, 4, 14		1, 2, 4, 5, 10
4	1.94 qd	7.2 (14), 4.5 (3α, 3β)	3α, 3β, 14	2α, 2β, 6, 14, 15	33.48 (t)	2, 3, 5, 6, 14
5					42.35 (q)	
6	5.40 dd	6.0 (13A), 3.2 (13B)		4, 15	85.95 (t)	4, 7, 11, 15
7					136.6 (q)	
8	4.77 dd	4.8 (9β), 1.8 (9α)	9α, 9β	9α, 9β	63.69 (t)	6, 7, 10, 11
9α	1.61 dt	14.5 (9β), 1.8 (8, 10)	8, 10	1β, 8, 10,	34.52 (s)	1, 5, 7, 10
9β	1.85 dt	14.5 (9α), 4.8 (8, 10)	8, 10	8, 10, 15		1, 5, 7, 8
10	1.67 m		1β, 9α, 9β	1β, 2β, 9α, 9β, 14	37.21 (t)	1, 5, 6, 15
11					132.82 (q)	
12A	4.15 d	12.8 (12B)	12B	12B	55.68 (s)	7, 8, 11
12B	4.40 d	12.8 (12A)	12A	12A		7, 8, 11, 13
13A	4.55 dd	12.5 (13B), 6.0 (6)	13B	13B	76.36 (s)	6, 7, 11, 12
13B	4.78 dd	12.5 (13A), 3.2 (6)	13A	13A		6, 7, 11
14	0.96 d	7.2 (4)	4	2β, 3β, 4, 10, 15	15.17 (p)	3, 4, 10
15	0.71 s			4, 6, 9β, 10, 14	17.78 (p)	4, 5, 6, 10

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

Table 5 Inhibitory activity of cell adhesion of metabolites

	Compound	IC_{50}/μM
Peribysin	A (**1**)	0.3
	B (**2**)	2.7
	C (**3**)	2.7
	D (**4**)	0.1
Herbimycin A	(Standard)	38.0

MHz for ^1H and ^{13}C, respectively, with TMS as an internal reference. ORD and CD spectra were recorded on a JASCO J-820 polarimeter. 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.) column. Analytical TLC was performed on silica gel precoated Merck aluminium sheets (DC-Alufolien Kieselgel 60 F254, 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 was separated from the sea hare *Aplysia kurodai* as reported previously.[5] The sea hare and the fungal strain were identified by Drs Y. Nabeshima (Osaka Prefectural Fisheries Experimental Station) and T. Ito (National Institute of Technology and Evaluation, Biological Resource Center). A voucher specimen of *A. kurodai* and *P. byssoides* has been deposited at Osaka University of Pharmaceutical Sciences and National Institute of Technology and Evaluation, respectively. The fungal strain was cultured at 27 °C for four weeks in a liquid medium (90 dm^3) containing malt extract 1%, glucose 1% and peptone 0.05% in artificial seawater adjusted to pH 7.5. As reported previously,[5] 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) columns. The MeOH–CH_2Cl_2 (1 : 99) eluate (214.6 mg) from silica gel column chromatography was purified by HPLC using MeOH–H_2O (9 : 1) as the eluent to afford **1** (57.8 mg), **2** (5.7 mg), **3** (28.6 mg) and **4** (22.8 mg).

Peribysin A 1. Obtained as a pale yellow oil, $[\alpha]_D$ −63.7 (*c* 4.28 in EtOH); λ_{max} (EtOH)/nm 233 (log ε/dm^3 mol^{-1} cm^{-1} 2.66); ν_{max} (liquid)/cm^{-1} 3347 (OH) and 1651 (C=C); *m*/*z* (EI) 253 (MH^+, 4.0%) and 137 (100) {*m*/*z* (HREI) Found: [M + H]$^+$, 253.1802. $C_{15}H_{25}O_3$ requires *M*, 253.1803}. ^1H and ^{13}C HMR data are listed in Table 1.

Peribysin B 2. Obtained as a pale yellow oil, $[\alpha]_D$ +42.9 (*c* 0.07 in EtOH); λ_{max} (EtOH)/nm 223 (log ε/dm^3 mol^{-1} cm^{-1} 2.68); ν_{max} (liquid)/cm^{-1} 3392 (OH); *m*/*z* (EI) 268 (M^+, 0.6%) and 109 (100) [*m*/*z* (HREI) Found: M^+, 268.1665. $C_{15}H_{24}O_4$ requires *M*, 268.1673]. ^1H and ^{13}C HMR data are listed in Table 2.

Peribysin C 3. Obtained as a pale yellow oil, $[\alpha]_D$ +31.5 (*c* 0.54 in EtOH); λ_{max} (EtOH)/nm 214 (log ε/dm^3 mol^{-1} cm^{-1} 3.58); ν_{max} (liquid)/cm^{-1} 1612 (C=C); *m*/*z* (EI) 234 (M^+, 24.4%) and 110 (100) [*m*/*z* (HREI) Found: M^+, 234.1619. $C_{15}H_{22}O_2$ requires *M*, 234.1619]. CD λ (*c* 5.2 × 10^{-4} mol dm^{-3} in EtOH)/nm 281 (Δε −0.3) and 226 (+2.0). ^1H and ^{13}C HMR data are listed in Table 3.

Peribysin D 4. Obtained as a pale yellow oil, $[\alpha]_D$ +4.6 (*c* 0.10 in EtOH); λ_{max} (EtOH)/nm 214 (log ε/dm^3 mol^{-1} cm^{-1} 3.56); ν_{max} (liquid)/cm^{-1} 1612 (C=C); *m*/*z* 234 (EI) (M^+, 66.5%) and 109 (100) [*m*/*z* (HREI) Found: M^+, 234.1618. $C_{15}H_{22}O_2$ requires *M*, 234.1619]. CD λ (*c* 2.5 × 10^{-4} mol dm^{-3} in EtOH)/nm 297 (Δε +0.1) and 264 (−0.2). ^1H and ^{13}C HMR data are listed in Table 4.

Cell-adhesion assay

This assay was carried out according to a modification of Miki's method using 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT)-labeled cells.[10] HUVEC cells (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 cells were stimulated with a solution of lipopolysaccharides (LPS, Sigma) in RPMI 1640 medium (Gibco) containing 10% FCS for 4 h in the presence of various concentrations of peribysins, and then MTT-labeled HL-60 cells were added and incubated for 40 min at 37 °C in 5% CO_2. Unbound cells were gently washed away with PBS containing 10% FCS. DMSO was added to lyse the adherent HL-60 cells. The absorbance at 540 nm was measured using a microplate reader (Model 450, BIO-RAD).

Acknowledgements

We thank Drs Y. Nabeshima (Osaka Prefectural Fisheries Experimental Station) and T. Ito (National Institute of Technology and Evaluation, Biological Resource Center) for the identification of the sea hare and the fungal strain, respectively. This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture, Japan.

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