JOURNAL OF NATURAL PRODUCTS

Note

pubs.acs.org/jnp

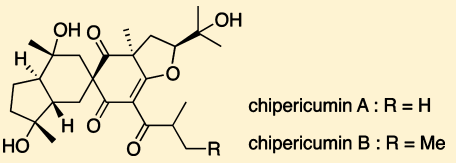
Prenylated Acylphloroglucinols, Chipericumins A−D, from *Hypericum chinense*[⊥]

Shuhei Abe, Naonobu Tanaka, and Jun'ichi Kobayashi*

Graduate School of Pharmaceutical Sciences, Hokkaido University, Sapporo 060-0812, Japan

Supporting Information

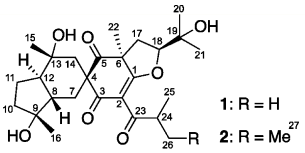
ABSTRACT: Two new tetracyclic prenylated acylphloroglucinols, chipericumins A (**1**) and B (**2**), were isolated from the roots of *Hypericum chinense*, together with two new tricyclic prenylated acylphloroglucinols, chipericumins C (**3**) and D (**4**). Their structures were elucidated by spectroscopic data. Chipericumins A−D (**1**−**4**) are prenylated acylphloroglucinols having a spiro skeleton with an acyl group, a methyl group, a C_5 unit, and a monoterpene moiety in common.

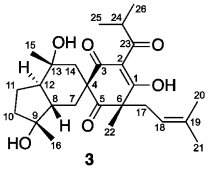


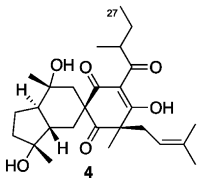
The plants of the genus *Hypericum* (family Clusiaceae) have been used as traditional remedies in several parts of the world.[1] These plants are known to contain various types of compounds such as naphthodianthrones, xanthones, flavonoids, and prenylated acylphloroglucinols. Among them, prenylated acylphloroglucinols have attracted much scientific interest because of their fascinating chemical structures and intriguing biological activities.[1,2] During a search for structurally interesting compounds from *Hypericum* spp., we have reported the isolation of prenylated acylphloroglucinols, petiolins A−D and J−M from *H. pseudopetiolatum* var. *kiusianum*[3] and yojironins C−I from *H. yojiroanum*.[4] We have also reported the isolation of prenylated xanthones, biyouxanthones A−D,[5] from the MeOH extract of the roots of *Hypericum chinense* Retz. Further investigation of this species has resulted in the isolation of four new prenylated acylphloroglucinols, chipericumins A−D (**1**−**4**). In this paper, we describe the isolation and structure elucidation of **1**−**4**.

The roots of *H. chinense* (2.52 kg, dried) were extracted with MeOH, and the extracts were partitioned successively with *n*-hexane, EtOAc, and H_2O. The EtOAc-soluble portions were subjected to passage over Toyopearl HW-40 (MeOH/H_2O), Sephadex LH-20 (MeOH), and silica gel (*n*-hexane/EtOAc) to give fractions containing prenylated acylphloroglucinols. The fractions were purified by ODS column chromatography (MeOH/H_2O), C_{18} HPLC (MeOH/H_2O), and silica gel HPLC (*n*-hexane/EtOAc and *n*-hexane/*i*-PrOH), to yield chipericumins A (**1**, 0.000035%), B (**2**, 0.000054%), C (**3**, 0.000090%), and D (**4**, 0.00023%).

Chipericumin A (**1**) was obtained as an optically active colorless amorphous solid {$[\alpha]^{25}_D$ +84.3 (*c* 0.29, MeOH)}. The molecular formula of **1**, $C_{26}H_{38}O_7$, was established by HRESIMS (*m*/*z* 485.2503 $[M + Na]^+$, Δ −0.7 mmu). The ^1H and ^{13}C NMR spectra (Table 1) showed the presence of a 2-methylpropanoyl group, an enol, two carbonyl groups, five sp^3 quaternary carbons, three sp^3 methines, five sp^3 methylenes, and five tertiary methyls. Among them, three sp^3 quaternary carbons {δ_C 79.1 (C-9), 73.0 (C-13), and 70.4 (C-19)} and a sp^3 methine {δ_C 91.9 (C-18)} were ascribed to those bearing







an oxygen atom. The gross structure of **1** was assigned as follows. A $^1H-^1H$ COSY correlation for H_2-17/H-18 and HMBC cross-peaks of H_3-20 to C-18, C-19, and C-21 suggested the presence of a 2-methylbutane-2,3-diol moiety (C-17−C-21) (Figure 1). The occurrence of a monoterpene moiety (C-7−C-16) was disclosed on the basis of several 2D NMR correlations, namely, H_2-7/H-8, H-8/H-12, H_2-10/H_2-11, and H_2-11/H-12 in the $^1H-^1H$ COSY spectrum and H_3-15 to C-12, C-13, and C-14, and H_3-16 to C-8, C-9, and C-10 in the HMBC spectrum. The chemical shifts of C-1−C-6 implied that the phloroglucinol moiety (C-1−C-6) consists of a ketone {δ_C 208.2 (C-5)}, a conjugated ketone {δ_C 197.9 (C-3)}, an enol {δ_C 178.5 (C-1) and 112.5 (C-2)}, and two sp^3 quaternary carbons {δ_C 60.2 (C-4) and 58.0 (C-6)}. The connectivities of C-6 to C-1, C-5, C-17, and C-22 were revealed by HMBC correlations for H_3-22 to C-1, C-5, C-6, and C-17, while connectivities between C-3, C-5, and the monoterpene moiety

Special Issue: Special Issue in Honor of Gordon M.Cragg

Received: September 15, 2011
Published: November 10, 2011

Table 1. ^{1}H and ^{13}C NMR Data for Chipericumins A (1) and B (2) in $CDCl_3$

	chipericumin A (**1**)		chipericumin B (**2**)	
position	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	178.5		178.7	
2	112.5		113.2	
3	197.9		198.4	
4	60.2		60.3	
5	208.2		208.3	
6	58.0		58.2	
7a	31.5	1.63, m	31.7	1.63, m
7b		1.71, dd (13.4, 3.4)		1.69, m
8	48.7	1.88, m	48.9	1.86, m
9	79.1		79.2	
10	40.2	1.79 (2H), m	40.3	1.76 (2H), m
11	21.9	1.86, 1.45, m	22.0	1.83, 1.44, m
12	51.7	1.91, m	51.7	1.89, m
13	73.0		73.1	
14a	46.9	2.20, d (14.4)	46.9	2.21, d (14.3)
14b		2.10, br d (14.4)		2.09, d (14.3)
15	21.7	1.10 (3H), s	21.7	1.09 (3H), s
16	26.6	1.27 (3H), s	26.7	1.26 (3H), s
17a	32.1	2.47, t (10.8)	32.2	2.43, t (10.9)
17b		1.95, dd (10.8, 5.5)		1.94, dd (10.9, 5.3)
18	91.9	4.61, dd (10.8, 5.5)	92.3	4.63, dd (10.9, 5.3)
19	70.4		70.5	
20	24.2	1.16 (3H), s	24.3	1.15 (3H), s
21	26.9	1.35 (3H), s	26.8	1.32 (3H), s
22	26.0	1.62 (3H), s	26.0	1.62 (3H), s
23	204.0		204.3	
24	39.9	3.19, sept (6.8)	46.5	3.00, qt (6.8, 6.8)
25	18.2	1.05 (3H), d (6.8)	15.8	1.07 (3H), d (6.8)
26	18.1	1.10 (3H), d (6.8)	26.2	1.63, 1.31, m
27			11.7	0.82 (3H), t (7.4)

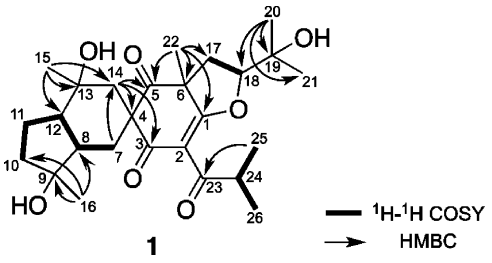


Figure 1. Selected 2D NMR correlations for chipericumin A (**1**).

(C-7 and C-14) via a spiro-carbon (C-4) were disclosed by HMBC cross-peaks of H_2-7 to C-14 and of H_2-14 to C-3, C-4, and C-5. Connectivities of C-1 to C-3 and a 2-methylpropanoyl group through C-2 were implied by the chemical shift of C-2 (δ_C 112.5).[3c,6] The presence of an ether linkage between C-1 and C-18 or C-19 was deduced by the unsaturation degree of **1**, taking the chemical shifts of C-1 (δ_C 178.5), C-18 (δ_C 91.9), and C-19 (δ_C 70.4) into consideration. In the deuterium-induced shift experiment of ^{13}C NMR measured in CD_3OD and CD_3OH, relatively large shifts ($\Delta\delta$ 0.1 ppm) were observed in C-9, C-13, and C-19, but not for C-18, suggesting that **1** has hydroxy groups at C-9, C-13, and C-19 and an ether linkage between C-1 and C-18. Thus, the gross structure of **1** was assigned as shown in Figure 1.

The relative configuration of **1** was assigned by ROESY NMR analysis (Figure 2). ROESY correlations for H-7a/H-14a,

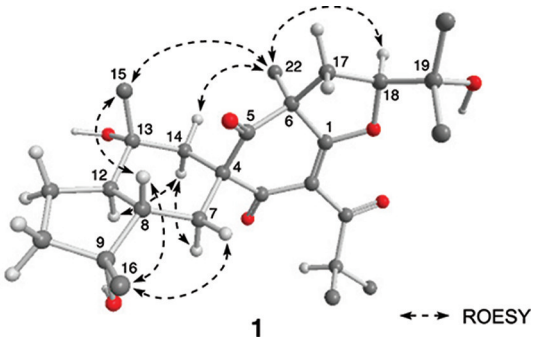


Figure 2. Selected ROESY correlations and relative configuration of chipericumin A (**1**) (protons of methyl groups are omitted).

H-12/H-14a, and H-8/H_3-15 indicated that H-7a, H-8, H-12, H-14a, and Me-15 have axial orientations. Thus, the cyclohexane ring (C-4, C-7, C-8, and C-12–C-14) adopts the chair conformation. The β-orientation for Me-16 was disclosed by the ROESY cross-peaks of H-7b/H_3-16 and H-8/H_3-16. ROESY correlations for H_3-15/H_3-22 and H-14b/H_3-22 indicated that Me-22 is present on the upper side of the cyclohexane ring, while the relative configurations of C-6 and C-18 were disclosed to be S* and S*, respectively, by a ROESY correlation for H-18/H_3-22. Accordingly, the relative configuration of **1** was assigned as shown in Figure 2.

Chipericumin B (**2**) was obtained as an optically active colorless amorphous solid {$[\alpha]^{25}_D$ +116.1 (c 0.46, MeOH)}. The HRESIMS of **2** revealed the molecular formula to be $C_{27}H_{40}O_7$ (m/z 499.2659 $[M + Na]^+$, Δ −0.7 mmu), larger by 14 mass units as compared with **1**. The ^{1}H and ^{13}C NMR data of **2** (Table 1) were quite similar to those of **1**, and signals for a 2-methylbutanoyl group in **2** [δ_H 3.00 (1H, qt, J = 6.8, 6.8 Hz), 1.63 and 1.31 (1H each, m), 1.07 (3H, d, J = 6.8 Hz), and 0.82 (3H, t, J = 7.4 Hz); δ_C 46.5, 26.2, 15.8, and 11.7] were discerned in place of the resonances of a 2-methylpropanoyl group in **1**. The 2-methylbutanoyl group in **2** was confirmed by analysis of the ^{1}H–^{1}H COSY and HMBC spectra (Figure 3).

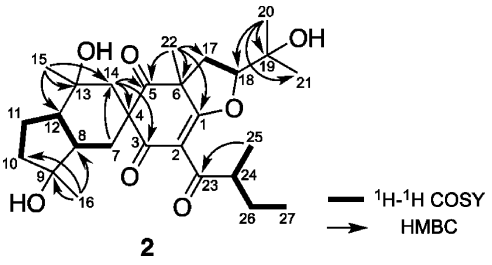


Figure 3. Selected 2D NMR correlations for chipericumin B (**2**).

The relative configurations for C-4, C-6, C-8, C-9, C-12, C-13, and C-18 were assigned as being the same as those of **1** on the basis of NOESY analysis, while the configuration of C-24 was not elucidated. Thus, the structure of chipericumin B was concluded as **2**.

Chipericumin C (**3**) was isolated as an optically active colorless amorphous solid {$[\alpha]^{25}_D$ +35.1 (c 0.33, MeOH)}. HRESIMS analysis indicated the molecular formula to be $C_{26}H_{38}O_6$ (m/z 445.2596 $[M - H]^-$, Δ 0.0 mmu). Analysis of the ^{1}H and ^{13}C NMR data for **3** (Table 2) implied this compound to be a prenylated acylphloroglucinol derivative having a monoterpene moiety similar to **1**, as well as a 2-

dx.doi.org/10.1021/np200741x | J. Nat. Prod. 2012, 75, 484–488

Table 2. ^{1}H and ^{13}C NMR Data for Chipericumins C (3) and D (4) in $CDCl_3$

	chipericumin C (3)		chipericumin D (4)	
position	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	200.1		199.4	
2	110.4		112.4	
3	197.4		196.4	
4	65.3		66.5	
5	207.0		207.9	
6	57.2		56.4	
7a	26.5	1.88, m	23.2	1.90, t (12.9)
7b		1.88, m		1.79, m
8	48.1	1.55, m	47.8	1.64, m
9	79.3		79.3	
10	39.9	1.76 (2H), m	39.5	1.78 (2H), m
11	21.6	1.77, 1.38, m	21.6	1.76, 1.35, m
12	51.9	1.77, m	52.0	1.75, m
13	73.3		73.4	
14a	48.4	1.45, d (13.7)	51.1	1.45, d (12.7)
14b		2.22, d (13.7)		2.36, dd (12.7, 1.6)
15	21.1	1.04 (3H), s	20.3	0.94 (3H), s
16	26.7	1.34 (3H), s	26.7	1.40 (3H), s
17a	35.4	2.72, dd (13.9, 8.8)	40.4	2.65, dd (13.3, 7.4)
17b		2.60, dd (13.9, 6.7)		2.38, dd (13.3, 7.4)
18	118.7	4.92, br t (7.7)	117.1	4.55, t (7.4)
19	136.1		137.3	
20	17.8	1.57 (3H), s	17.5	1.38 (3H), s
21	25.8	1.62 (3H), s	25.8	1.49 (3H), s
22	26.2	1.33 (3H), s	22.4	1.49 (3H), s
23	205.2		205.1	
24	33.9	3.43, sept (6.8)	41.9	3.16, m
25	18.2	1.15 (3H), d (6.8)	19.5	1.22 (3H), d (6.8)
26	20.6	1.24 (3H), d (6.8)	25.3	1.78, 1.44, m
27			12.3	0.83 (3H), t (6.9)
OH-1		18.19, s		18.39, s

methylpropanoyl group, a prenyl group, and a methyl group. The chemical shifts of C-1−C-6 were consistent with the phloroglucinol moiety of **3** being characterized by a ketone {δ_C 207.0 (C-5)}, a conjugated ketone {δ_C 197.4 (C-3)}, an enol {δ_C 200.1 (C-1) and 110.4 (C-2)}, and two sp^3 quaternary carbons {δ_C 65.3 (C-4) and 57.2 (C-6)}. The characteristic downfield shifted signal of OH-1 (δ_H 18.19) suggested the presence of a hydrogen bond between the proton of OH-1 and an oxygen atom of a carbonyl group,[6] implying that a 2-methylpropanoyl group is connected to C-2. This inference was supported by HMBC correlations for OH-1 to C-1 and C-2 as well as the chemical shift of C-2 (δ_C 110.4). HMBC correlations for H_3-22 to C-1, C-5, C-6, and C-17 implied that C-6 is connected to C-1, C-5, the methyl group (C-22), and the prenyl group (C-17). In addition, HMBC cross-peaks of H_2-7 to C-14 and of H_2-14 to C-3, C-4, and C-5 indicated the connectivities between C-3, C-5, C-7, and C-14 via C-4. Thus, the gross structure of **3** was elucidated as shown in Figure 4.

NOESY correlations for H-7a/H-14a, H-12/H-14a, H-8/H_3-15, H-8/H_3-16, and H-7b/H_3-16 suggested the relative configuration of the monoterpene moiety of **3** to be the same as that of **1** (Figure 5). Furthermore, NOESY cross-peaks of H_3-15/H-24 and H-14b/H-18 revealed the relative configurations of C-4 and C-6 to be *S** and *S**, respectively.

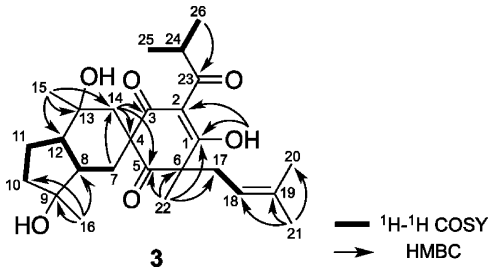


Figure 4. Selected 2D NMR correlations for chipericumin C (**3**).

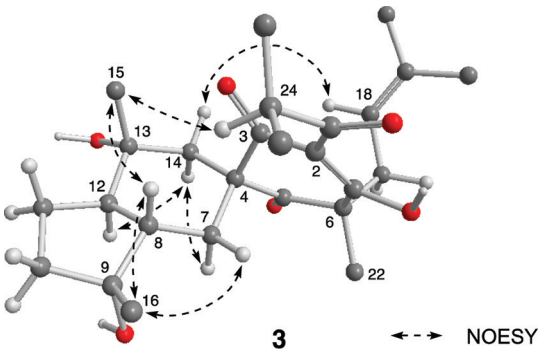


Figure 5. Selected NOESY correlations and relative configuration of chipericumin C (**3**) (protons of methyl groups are omitted).

Therefore, the relative configuration of **3** was assigned as shown in Figure 5.

Chipericumin D (**4**) was isolated as an optically active colorless amorphous solid {$[\alpha]^{21}_D$ +67.4 (*c* 0.33, MeOH)}. The ^{1}H and ^{13}C NMR spectra of **4** resembled those of **3** and showed the presence of a 2-methylbutanoyl group in place of a 2-methylpropanoyl group in **3**. These NMR observations suggested that a 2-methylpropanoyl group in **3** is replaced by a 2-methylbutanoyl group in **4**. This was supported by analysis of the $^{1}H-^{1}H$ COSY and HMBC spectra and from the molecular formula of $C_{27}H_{40}O_6$, which was revealed by HRESIMS (*m*/*z* 459.2754 $[M - H]^-$, Δ +0.2 mmu). Subtle differences between the chemical shifts for C-7, C-14, C-17, and C-22 of **4** and those found in **3** implied that the relative configuration of **3** is different from that of **4**. The relative configurations of the monoterpene moiety and C-4 in **4** were assigned to be the same as those of **3** by NOESY analysis (Figure 6). A NOESY cross-peak between H-7b/H-18 suggested that C-7 and the prenyl group at C-6 are oriented on the same side of the molecule. The configuration of C-24 still remains to be assigned. Thus, the structure of chipericumin D was concluded as **4**.

In the CD spectrum, chipericumins A (**1**) and B (**2**) gave closely correlated Cotton effects (Figure 7). These observations suggested that the configuration at the C-4 spiro-center of **1** is the same as that of **2**.[7] On the other hand, the CD spectrum of chipericumin C (**3**) resembled that of chipericumin D (**4**), implying that **3** and **4** shared the same configuration at C-4, which seems to be opposite of those found in **1** and **2**.

Chipericumins A (**1**) and B (**2**) are prenylated acylphloroglucinols with tetracyclic ring systems, and chipericumins C (**3**) and D (**4**) are tricyclic prenylated acylphloroglucinols. They have a spiro skeleton with an acyl group, a methyl group, a C_5 unit, and a monoterpene moiety in common. Structurally related acylphloroglucinols fused with a monoterpene moiety by a spiro-linkage, such as harrisotones A−E,[7] hyperielliptone

dx.doi.org/10.1021/np200741x | *J. Nat. Prod.* 2012, 75, 484−488

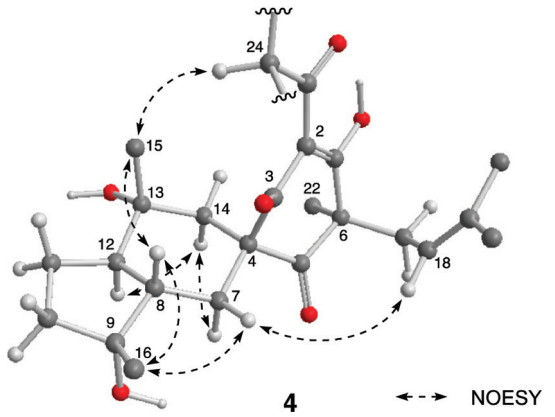


Figure 6. Selected NOESY correlations and relative configuration of chipericumin D (**4**) (C-25−C-27 and protons of methyl groups are omitted).

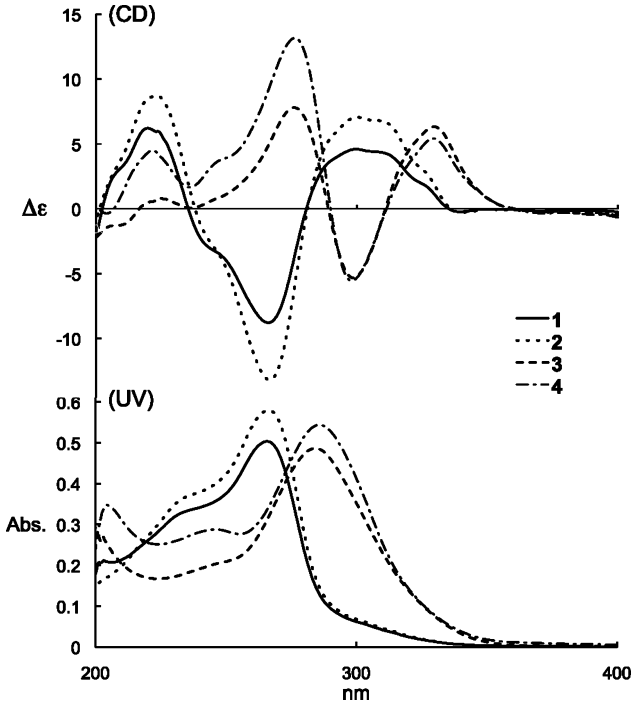


Figure 7. CD and UV spectra of chipericumins A−D (**1**−**4**).

HB,[8] tomoeones A−H,[6] and biyouyanagiol,[9] have been so far found in the plants belonging to *Hypericum* (Clusiaceae)[6,8,9] and *Harrisonia* (Simaroubaceae).[7] A biogenetic pathway for chipericumins A (**1**) and B (**2**) is proposed as shown in Scheme 1. Thus, epoxidation of the double bonds of chinesins II and I, prenylated acylphloroglucinols previously isolated from the same plant,[10] seems to occur and is followed by intramolecular cyclizations to generate chipericumins A (**1**) and B (**2**), respectively.

Chipericumins A−D (**1**−**4**) did not show cytotoxicity against murine lymphoma L1210 cells and human epidermoid carcinoma KB cells (all IC_{50} >10 μg/mL) in vitro and did not exhibit antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Micrococcus luteus* (all MIC > 32 μg/mL) or against *Aspergillus niger*, *Candida albicans*, *Cryptococcus neoformans*, and *Trichophyton mentagrophytes* (all IC_{50} > 32 μg/mL).[11]

EXPERIMENTAL SECTION

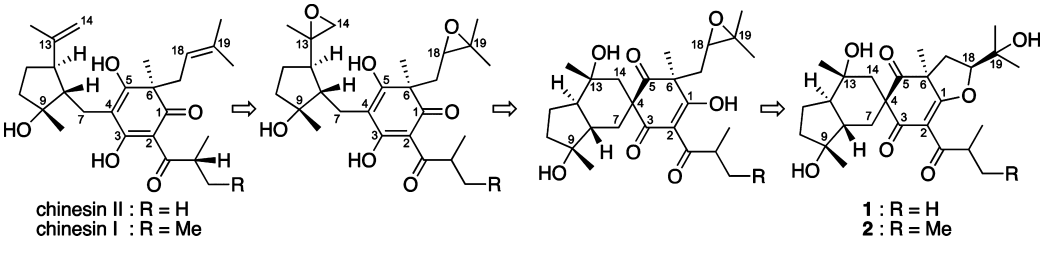
General Experimental Procedures. Optical rotations were recorded using a JASCO P-1030 digital polarimeter. IR, UV, and CD spectra were recorded on JASCO FT/IR-230, Shimadzu UV-1600PC, and JASCO J-720 spectrophotometers, respectively. NMR spectra were measured by a Bruker AMX-600 spectrometer. The 7.26 and 77.0 ppm resonances of residual $CHCl_3$ were used as internal references for ^1H and ^{13}C NMR spectra, respectively. HRESIMS and ESIMS were recorded on a Thermo Scientific Exactive spectrometer.

Plant Material. *Hypericum chinense* was cultivated at the botanical garden of the University of Tokushima and collected in January 2006. Herbarium specimens were deposited at the Experimental Station for Medicinal Plants Studies, Hokkaido University (specimen number: UTP98014).

Extraction and Isolation. The roots of *H. chinense* (2.52 kg, dry) were extracted with MeOH (3 × 10 L), and the extract (253 g) was partitioned successively with *n*-hexane (3 × 1 L), EtOAc (3 × 1 L), and H_2O (1 L). A part (15.5 g) of the EtOAc-soluble portion (41.4 g) was subjected to successive chromatography over a Toyopearl HW-40 column (MeOH/H_2O, 50:50 → 100:0), a Sephadex LH-20 column (MeOH), and a silica gel column (*n*-hexane/EtOAc, 80:20 → 0:100) to give seven fractions (fr. 1−7). Fr. 5 was purified by silica gel HPLC (Mightysil Si60, Kanto Chemical Co., Inc., 10 × 250 mm; flow rate 3.5 mL/min; UV detection at 254 nm; eluent *n*-hexane/EtOAc, 75:25) and C_{18} HPLC (Capcell Pak C_{18}MG, Shiseido, 10 × 250 mm; flow rate 2.0 mL/min; UV detection at 254 nm; eluent MeOH/H_2O/TFA, 90:10:0.05) to yield chipericmin C (**3**, 2.3 mg, 0.000090%). Fr. 6 was separated using an ODS column (MeOH/H_2O, 60:40 → 100:0) to give four fractions (fr. 6.1−6.4). Fr. 6.1 was purified by C_{18} HPLC (Capcell Pak C_{18}MG, 10 × 250 mm; flow rate 2.0 mL/min; UV detection at 254 nm; eluent MeOH/H_2O, 65:35) and silica gel HPLC (Mightysil Si60, 10 × 250 mm; flow rate 2.5 mL/min; UV detection at 254 nm; eluent *n*-hexane/*i*-PrOH, 85:15) to yield chipericumins A (**1**, 0.9 mg, 0.000035%) and B (**2**, 1.4 mg, 0.000054%). Purification of fr. 6.3 by silica gel HPLC (Mightysil Si60, 20 × 250 mm; flow rate 5.0 mL/min; UV detection at 254 nm; eluent *n*-hexane/EtOAc, 45:55) gave chipericumin D (**4**, 5.8 mg, 0.00023%).

Chipericumin A (1): colorless amorphous solid; $[\alpha]^{25}_D$ +84.3 (*c* 0.29, MeOH); UV (MeOH) λ_{max} 234 (ε 7660), 265 (11 610) nm; CD (MeOH) λ_{ext} 220 (Δε +6.2), 267 (−8.8), 300 (+4.6) nm; IR (film) ν_{max} 3422, 1714, 1693, 1618 cm^{-1}; ^1H and ^{13}C NMR data (Table 1); ESIMS *m*/*z* 485 $[M + Na]^+$; HRESIMS *m*/*z* 485.2503 $[M + Na]^+$ (calcd for $C_{26}H_{38}O_7Na$, 485.2510).

Scheme 1. Possible Biogenetic Pathway for Chipericumins A (1) and B (2)



dx.doi.org/10.1021/np200741x | J. Nat. Prod. 2012, 75, 484−488

Chipericumin B (2): colorless amorphous solid; $[\alpha]^{25}_{D}$ +116.1 (*c* 0.46, MeOH); UV (MeOH) λ_{max} 234 (ε 8730), 265 (13 750) nm; CD (MeOH) λ_{ext} 224 ($\Delta\varepsilon$ +8.7), 267 (−13.2), 300 (+7.1) nm; IR (film) ν_{max} 3446, 1717, 1696, 1615 cm^{-1}; ^{1}H and ^{13}C NMR data (Table 1); ESIMS *m/z* 499 $[M + Na]^+$; HRESIMS *m/z* 499.2659 $[M + Na]^+$ (calcd for $C_{27}H_{40}O_7Na$, 499.2666).

Chipericumin C (3): colorless amorphous solid; $[\alpha]^{25}_{D}$ +35.1 (*c* 0.33, MeOH); UV (MeOH) λ_{max} 247 (ε 4550), 284 (10 860) nm; CD (MeOH) λ_{ext} 225 ($\Delta\varepsilon$ +0.8), 277 (+7.8), 299 (−5.3), 330 (+6.3) nm; IR (film) ν_{max} 3361, 1722, 1675 cm^{-1}; ^{1}H and ^{13}C NMR data (Table 2); ESIMS *m/z* 445 $[M - H]^-$; HRESIMS *m/z* 445.2596 $[M - H]^-$ (calcd for $C_{26}H_{37}O_6$, 445.2596).

Chipericumin D (4): colorless amorphous solid; $[\alpha]^{21}_{D}$ +67.4 (*c* 0.33, MeOH); UV (MeOH) λ_{max} 246 (ε 6620), 286 (12 500) nm; CD (MeOH) λ_{ext} 222 ($\Delta\varepsilon$ +4.5), 276 (+13.2), 298 (−5.5), 329 (+5.4) nm; IR (film) ν_{max} 3436, 1717, 1672 cm^{-1}; ^{1}H and ^{13}C NMR data (Table 2); ESIMS *m/z* 459 $[M - H]^-$; HRESIMS *m/z* 459.2754 $[M - H]^-$ (calcd for $C_{27}H_{39}O_6$, 459.2752).

Cytotoxicity Testing. Human epidermoid carcinoma (KB) and murine leukemia L1210 cells were cultured in an incubator at 37 °C for 48 h in 100 μL of medium containg various concentrations of test compounds dissolved in 1% DMSO. The IC_{50} values were obtained by plotting the logarithm of the concentration of the test compound versus the growth rate of the treated cells. Paclitaxel was used as positive control {IC_{50} 4.5 nM (KB) and 120 nM (L1210)}.

Antimicrobial Testing. Antimicrobial assay of **1**−**4** against *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Micrococcus luteus*, *Aspergillus niger*, *Candida albicans*, *Cryptococcus neoformans*, and *Trichophyton mentagrophytes* was carried out as previously described.[11]

■ ASSOCIATED CONTENT

Supporting Information

The ^{1}H and ^{13}C NMR spectra of chipericumins A−D (**1**−**4**) are available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Tel: +81-11-706-3239. Fax: +81-11-706-4989. E-mail: jkobay@pharm.hokudai.ac.jp.

■ ACKNOWLEDGMENTS

We thank Prof. Y. Takaishi, Graduate School of Pharmaceutical Sciences, University of Tokushima, for collection of the plant, Prof. T. Gonoi, Medicinal Mycology Research Center, Chiba University, for antimicrobial evaluation, and S. Oka and A. Tokumitsu, Equipment Management Center, Hokkaido University, for measurements of the HRESIMS. This work was partly supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

■ DEDICATION

⊥Dedicated to Dr. Gordon M. Cragg, formerly Chief, Natural Products Branch, National Cancer Institute, Frederick, Maryland, for his pioneering work on the development of natural product anticancer agents.

■ REFERENCES

(1) Avato, P. In *Studies in Natural Products Chemistry*; Atta-ur-Rahman., Ed.; Elsevier Science B.V.: Amsterdam, 2005; Vol. *30*, pp 603−634.

(2) (a) Nahrstedt, A.; Butterweck, V. *Pharmacopsychiatry* **1997**, *30*, 129−134. (b) Singh, I. P.; Bharate, S. B. *Nat. Prod. Rep.* **2006**, *23*, 558−591. (c) Ciochina, R.; Grossman, R. B. *Chem. Rev.* **2006**, *106*, 3963−3986.

(3) (a) Tanaka, N.; Kubota, T.; Ishiyama, H.; Araki, A.; Kashiwada, Y.; Takaishi, Y.; Mikami, Y.; Kobayashi, J. *Bioorg. Med. Chem.* **2008**, *16*, 5619−5623. (b) Tanaka, N.; Kubota, T.; Ishiyama, H.; Kashiwada, Y.; Takaishi, Y.; Ito, J.; Mikami, Y.; Shiro, M.; Kobayashi, J. *Heterocycles* **2009**, *79*, 917−924. (c) Tanaka, N.; Otani, M.; Kashiwada, Y.; Takaishi, Y.; Shibazaki, A.; Gonoi, T.; Shiro, M.; Kobayashi, J. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 4451−4455.

(4) (a) Mamemura, T.; Tanaka, N.; Shibazaki, A.; Gonoi, T.; Kobayashi, J. *Tetrahedron Lett.* **2011**, *52*, 3575−3578. (b) Tanaka, N.; Mamemura, T.; Shibazaki, A.; Gonoi, T.; Kobayashi, J. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 5393−5397.

(5) Tanaka, N.; Mamemura, T.; Abe, S.; Imabayashi, K.; Kashiwada, Y.; Takaishi, Y.; Suzuki, T.; Takebe, Y.; Kubota, T.; Kobayashi, J. *Heterocycles* **2010**, *80*, 613−621.

(6) Hashida, W.; Tanaka, N.; Kashiwada, Y.; Sekiya, M.; Ikeshiro, Y.; Takaishi, Y. *Phytochemistry* **2008**, *69*, 2225−2230.

(7) Yin, S.; Chen, X.; Su, Z.-S; Yang, S.-P.; Fan, C.-Q.; Ding, J.; Yue, J.-M. *Tetrahedron* **2009**, *65*, 1147−1152.

(8) Wu, C.-C.; Yen, M.-H.; Yang, S.-C.; Lin, C.-N. *J. Nat. Prod.* **2008**, *71*, 1027−1031.

(9) Tanaka, N.; Kashiwada, Y.; Kim, S.-Y.; Hashida, W.; Sekiya, M.; Ikeshiro, Y.; Takaishi, Y. *J. Nat. Prod.* **2009**, *72*, 1447−1452.

(10) Nagai, M.; Tada, M. *Chem. Lett.* **1987**, *16*, 1337−1340.

(11) Nagai, H.; Mikami, Y.; Yazawa, K.; Gonoi, T.; Yasumoto, T. *J. Antibiot.* **1993**, *46*, 520−522.