Sundaicumones A and B, Polyprenylated Acylphloroglucinol Derivatives from *Calophyllum sundaicum* with Weak Activity against the Glucocorticoid Receptor

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Bioassay-directed fractionation using a glucocorticoid receptor assay led to the isolation of two new, weakly active polyprenylated acylphloroglucinol derivatives, sundaicumones A (**1**) and B (**2**), from the leaves of *Calophyllum sundaicum* collected in Singapore. The structures of **1** and **2**, which were established by spectroscopic methods, contain a 3-substituted hexanoic acid unit not previously reported in other polyprenylated acylphloroglucinols.

Polyprenylated acylphloroglucinols are a class of biologically active secondary metabolites isolated from the plant family Clusiaceae (Guttiferae), predominantly from the genera *Hypericum*,[1−3] *Garcinia*,[4−10] and *Clusia*[10−13] but also from *Symphonia*,[10] *Allanblackia*,[14] and *Calophyllum*.[15] Polyprenylated acylphloroglucinols also have been isolated from Cuban bee propolis and are thought to be present through transfer from the floral resin of *Clusia rosea*.[16,17] The best known example of a polyprenylated acylphloroglucinol is hyperforin (**3**),[1,2] a metabolite of *Hypericum perforatum* (St. John's Wort), which has been reported to have antidepressant[18] and in vitro and in vivo anticancer activity.[19] Other biological activities ascribed to polyprenylated acylphloroglucinols include HIV inhibition,[10,14] cytotoxic effects against cancer cell lines,[6,7,9,17,19] anti-inflammatory,[5] choline acetyltransferase inhibition,[4] and as ligand binders of the liver-X receptor.[8]

The glucocorticoid receptor (GR) belongs to the superfamily of ligand-activated transcription factors, the nuclear hormone receptors, which along with other steroid receptors affect the body through regulation of gene transcription.[20] The GR agonist complex binds and inhibits pro-inflammatory transcription factors such as nuclear factor-κB and activation protein-1, resulting in anti-inflammatory effects.[21,22] Synthetic glucocorticoids such as dexamethasone and prednisolone have been used since the 1960s to treat chronic inflammatory diseases but have side effects due to inhibition of other steroid receptors.[23] A reporter gene assay was developed to identify novel GR agonists as anti-inflammatory therapy for asthma and other airway diseases.

Screening of our natural product extract library in the GR assay identified an active extract from the leaves of *Calophyllum sundaicum* P.F. Stev. (Clusiaceae). Bioassay-guided isolation using a modified Kupchan solvent partition scheme[24] and preparative C_{18} reversed-phase HPLC gave two polyprenylated acylphloroglucinol derivatives, sundaicumones A (**1**) and B (**2**), which showed EC_{50} values of 173 and 75 μM, respectively, in the GR assay. The GR activity of these compounds was too weak to warrant any further biological investigation. Dexamethasone was used as a control in the GR assay and had an EC_{50} of 1.0 ± 0.6 nM.

Sundaicumone A (**1**) was obtained as a colorless oil and had a molecular formula of $C_{32}H_{50}O_9$ based on its HRESIMS. The ^{1}H NMR spectrum of **1** (Table 1) displayed signals for six methyl singlet resonances at δ_H 0.90, 0.96, 1.01, 1.08, 1.11, and 1.21, one methyl doublet at δ_H 0.98, and two methyl triplets at δ_H 0.70 and 0.81. The ^{13}C and multiplicity-edited HSQC NMR spectra of **1** showed nine CH_3, seven CH_2, five CH, and 11 quaternary carbons, six of which were located at low field (δ_C 208.5, 205.1, 192.2, 175.3, 174.0, and 116.5). Analysis of the COSY, TOCSY, HSQC, and HMBC NMR data indicated the presence of 2-methyl-1-oxobutyl (C-16 to C-20), 3-substituted-hexanoic acid (C-1′ to C-6′), 2-oxygenated-3-hydroxy-3-methylbutyl (C-2, C-3, and C-13 to C-15), and a dihydroxylated monoterpene (C-9 to C-11 and C-21 to C-27) moiety, which were attached to C-8, C-6, C-1/C-5, and C-1/C-8 of the acylphloroglucinol core, respectively. These data suggested that the structure of **1** is similar to garsubellin B (**4**) except for the 3-substituted hexanoic acid moiety at C-6 in **1** instead of an isoprene moiety.[4] A total synthesis of (±)-garsubellin A (**5**) was reported recently, which confirmed the structure and relative stereochemistry originally proposed.[25] Although H-17 did not exhibit a correlation to C-8 in the HMBC spectrum of **1**, the chemical shift of C-8 (δ_C 82.2) was consistent with that assigned in garsubellins B (**4**) and A (**5**).[4] Therefore, the planar structure of **1** was determined as shown and attention was focused on the compound's relative stereochemistry. In their paper on the structures of the sampsoniones, Hu and Sim discussed an empirical rule that can be used to establish the axial or equatorial orientation of C-10 side-chains in acylphloroglucinols.[3] In **1**, the C-10 side-chain was assigned as having an equatorial orientation due to the ^{13}C NMR resonances of the C-9 *gem*-dimethyl groups (C-21, δ 16.2 and C-22, δ 22.3) and the ^{1}H NMR resonances and coupling constants of H-11$_\beta$ (δ 1.53, dd, J = 13.8, 12.0 Hz) and H-11$_\alpha$ (δ 2.19, dd, J = 13.8, 4.4 Hz).[2−4,10] Furthermore, ROESY correlations between H-11$_\beta$ and H$_3$-21 confirmed the assigned relative stereochemistry

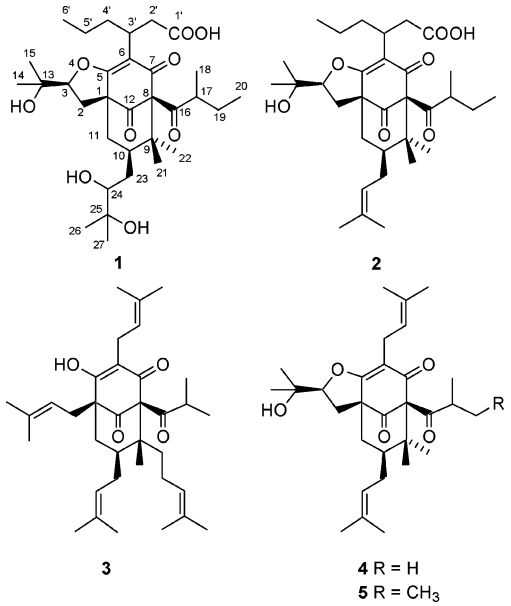

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Table 1. NMR Spectroscopic Data of Sundaicumones A (**1**) in DMSO-d_6 and B (**2**) in $CDCl_3$

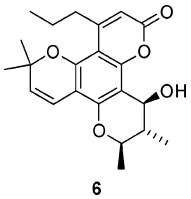
	1				**2**	
no.	^{13}C δ	^{1}H δ m J (Hz)	gHMBC (H to C)	ROESY/1D-ROESY	^{13}C δ^a	^{1}H δ m J (Hz)
1	60.0				59.9	
2	28.9	Hα: 1.84 dd 5.6, 12.9	C-1, 5, 11	2β, 3	30.5	Hα: 1.76 dd 6.2, 13.3
		Hβ: 2.46 dd 10.6, 12.9	C-1, 3, 11, 12, 13	2α		Hβ: 2.80 dd 10.2, 13.3
3	90.7	4.70 dd 5.6, 10.6	C-14, 15	2α, 14, 15, 11α	90.6	4.58 dd 6.2, 10.2
4						
5	175.3				175.6	
6	116.5				116.4	
7	192.2				192.1	
8	82.4				82.2	
9	46.3				46.0	
10	37.0	1.83 m	C-9, 21, 23	11α, 22, 23, 24	44.5	1.64 m
11	37.5	Hα: 2.19 dd 4.4, 13.8	C-1, 2, 5, 9, 10, 12, 23	3, 10, 11β, 24	40.1	Hα: 2.02 m
		Hβ: 1.53 dd 12.0, 13.8	C-1, 2, 5, 10, 23	11α, 21		Hβ: 1.51 m
12	205.1				204.7	
13	69.2				69.3	
14	25.9	1.21 s	C-3, 13, 15	3	28.8	1.48 s
15	25.3	1.11 s	C-3, 13, 14	3	24.7	1.15 s
16	208.5				208.5	
17	47.6	1.75 m	C-16, 18, 19, 20		49.4	1.81 m
18	16.3	0.98 d 6.5	C-16, 17, 19		17.8	1.10 d 6.5
19	27.0	Ha:1.11 m	C-16, 17, 18, 20		27.0	Ha: 1.32 m
		Hb: 1.54 m				Hb: 1.68 m
20	10.7	0.70 t 7.4	C-17, 19		11.8	0.80 t 7.5
21	16.2	0.90 s	C-8, 9, 10, 22	11β	17.0	1.27 s
22	22.3	1.08 s	C-8, 9, 10, 21	10	23.8	1.05 s
23	28.9	Ha:1.13 m	C-9, 24	10	27.6	Ha:1.65 m
		Hb: 1.31 m				Hb: 2.18 m
24	73.9	3.08 br d 10.8	C-10, 23, 25, 26, 27	10, 11α, 26	123.5	4.96 t 7.2
25	71.6				132.2	
26	26.5	1.01 s	C-24, 25, 27		26.9	1.70 s
27	24.6	0.96 s	C-24, 25, 26		18.6	1.57 s
1′	174.0				173.7	
2′	37.7	2.49 m	C-1′, 3′, 4′, 6	3′, 4′a, 4′b	38.1	Ha: 2.43 dd 3.3, 16.3
						Hb: 2.92 dd 11.9, 16.3
3′	30.8	3.30 m	C-2′, 4′, 5, 6, 7	2′	34.1	3.35 m
4′	33.9	Ha: 1.39 m	C-2′, 3′, 5′, 6′	2′	35.4	Ha: 1.54 m
		Hb: 1.71 m	C-2′, 3′, 5′, 6′, 6	2′		Hb: 2.02 m
5′	20.5	Ha: 1.08 m			20.5	1.29 m
		Hb: 1.16 m				
6′	14.0	0.81 t 7.2	C-4′, 5′		14.8	0.92 t 7.3

[a] ^{13}C NMR chemical shifts obtained from the HSQC and HMBC NMR spectra.

about C-10, while ROESY correlations between H-3 and H-11$_\alpha$ allowed the relative stereochemistry at C-3 to be assigned.

Sundaicumone B (**2**) was obtained as an unstable, colorless oil and was assigned a molecular formula of $C_{32}H_{50}O_9$ based on its HRESIMS. The NMR data of **2** were closely related to those of **1**, with the only difference being a 3-methyl-2-butenyl side-chain at C-10 in **2** in place of the 2,3-dihydroxyl-3-methylbutyl side-chain in **1**. Hence, the structure and relative stereochemistry of **2** were determined as shown.

Although sundaicumones A (**1**) and B (**2**) are closely related to other polyprenylated acylphloroglucinols, the 3-substituted hexanoic acid unit has not been previously reported in this series. However, the 3-substituted hexanoic acid unit has been found previously in apetalic acid,[26] isoapetalic acid,[27] calolongic acid,[28,29] isocalolongic acid,[29] recedensic acid,[28] brasiliensic acid,[30] isobrasiliensic acid,[30] and inocalophyllin B.[31] The 3-substituted hexanoic acid also is present as part of the coumarin ring in naturally occurring HIV-1 inhibitor calanolide A (**6**),[32] which is currently in phase II clinical evaluation in combination with other antiviral agents.[33]



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Experimental Section

General Experimental Procedures. Optical rotations were recorded on a JASCO DIP-1000 digital polarimeter. UV spectra were scanned on a Pharmacia Biotech Ultrospec 2000 and IR spectra on a Perkin-Elmer BioRad FT-IR spectrophotometer. NMR spectra were collected on a Bruker Avance DRX-500 NMR spectrometer, using 5 mm BBI (^1H, G-COSY, multiplicity-edited G-HSQC, G-HMBC, G-TOCSY, and G-ROESY spectra) or BBO (^{13}C spectra) probeheads equipped with z-gradients. Spectra were calibrated to residual protonated solvent signals. HRESIMS values were collected on an Applied Biosystems Mariner TOF mass spectrometer, using sodium trifluoroacetate as an internal standard for both positive- and negative-ionization modes. Preparative HPLC was performed on a Gilson system complete with UniPoint software, 170 DAD detector, dual 306 pumps, 811C dynamic mixer, Gilson 202 fraction collector, and a Rheodyne 7125 injector with a 5 mL injection loop.

Plant Material. The leaves of *C. sundaicum* P.F. Stev. (Clusiaceae) were collected in the Upper Pierce Reservoir, Singapore, in September 1994 and identified by Haji Sidek. A voucher specimen (CNPR 57) has been deposited at the Singapore Botanic Gardens herbarium.

Extraction and Isolation. Dried and milled leaves (46.9 g) were extracted with 1:1 CH_2Cl_2−MeOH (2 × 500 mL) and dried by rotary evaporation. The crude extract (8.1 g) was partitioned between 90% aqueous MeOH (1 L) and hexane (1 L), and the ratio of MeOH to H_2O was adjusted from 9:1 to 7:3 by addition of H_2O and further partitioned with CH_2Cl_2 (0.5 L × 2). The active CH_2Cl_2 fraction was separated by reversed-phase HPLC (Waters PrepLC 25 mm Module C_{18}, 25 × 100 mm) to yield sundaicumone A (**1**) (1.5 mg) and sundaicumone B (**2**) (1.2 mg).

Sundaicumone A (1): colorless oil; $[\alpha]_D^{25}$ +52 (*c* 0.2, EtOH); UV (EtOH) λ_{max} (log ϵ) 207 (4.67), 271 (4.90) nm; IR (film) ν_{max} 3442, 1729, 1620 cm^{-1}; NMR data, see Table 1; (−)-HRESIMS *m*/*z* 577.3415 (calcd for $C_{32}H_{50}O_9$, 577.3377).

Sundaicumone B (2): colorless oil; $[\alpha]_D^{25}$ +48 (*c* 0.1, EtOH); UV (EtOH) λ_{max} (log ϵ) 207 (3.89), 278 (3.85) nm; IR (film) ν_{max} 3440, 1725, 1618 cm^{-1}; NMR data, see Table 1; (−)-HRESIMS *m*/*z* 543.3290 (calcd for $C_{32}H_{48}O_7$, 543.3322).

Biological Assay. A 5 μL aliquot of each test compound in 12.5% DMSO was added to the wells of a Nunc 384-well white plate with a clear bottom. A549 cells (human lung carcinoma cell) transfected with a GRE promoter/Renilla luciferase reporter construct were seeded in 45 μL of Dulbecco's modified Eagle's medium containing 10% heat-inactivated fetal bovine serum, 2 mM l-glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin, at a final density of 10 000 cells/well. The plate was incubated for 24 h at 37 °C in a humidified 5% CO_2 incubator, and 20 μL of 20 μM coelenterazine was added to the wells. Following a 2 h incubation in the dark, ligand binding to the GR was determined by measuring luminescence in a microplate luminometer (Luminoskan Ascent, Labsystems) using a 600 ms/well integration time.

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