Phenolic Glycosides from the Filamentous Fungus *Acremonium* sp. BCC 14080

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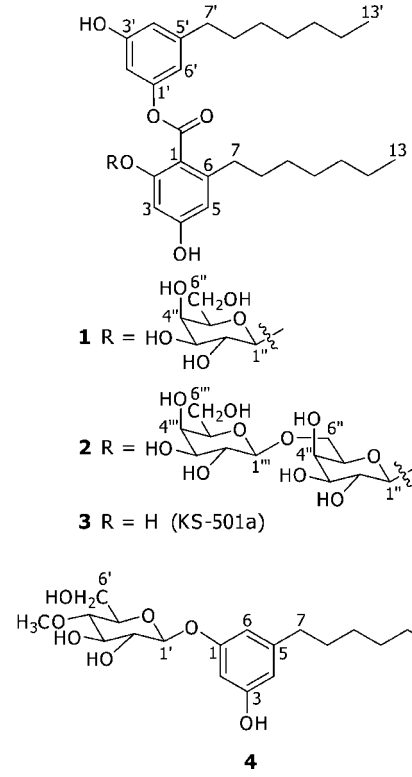
New phenolic mono- and digalactopyranosides (**1** and **2**), their aglycone KS-501a (**3**), and a new phenolic 4-*O*-methylglucopyranoside (**4**) were isolated from the filamentous fungus *Acremonium* sp. BCC 14080. Structures of these compounds were elucidated by extensive MS and NMR spectroscopic analyses. Compound **1** displayed anti-HSV-1 activity with an IC_{50} value of 7.2 μM. Compound **3** exhibited activity against *Plasmodium falciparum* K1 with an IC_{50} value of 9.9 μM.

Fungi in the genus *Acremonium* have been isolated from plants,[1,2] soil,[3,4] and marine organisms.[5,6] They are known to produce various novel secondary metabolites, some that have biological activities such as antitumor pentadecapeptides (efrapeptins),[6] antibiotic and antitumor pentacyclic polyketides (UCS1025A and B),[7] an antibacterial spirobenzofuran,[8] and plant growth inhibitors (acremolactones).[9] As part of our research program on bioactive constituents from Thai microorganisms,[10] we describe herein the isolation of two new depsides, **1** and **2**, containing one and two galactopyranose moieties, respectively, together with their aglycone, KS-501a (**3**),[11] and a new phenolic 4-*O*-methylglucopyranoside (**4**) from *Acremonium* sp. BCC 14080. Biological assays including antiplasmodial, cytotoxic, and anti-HSV-1 activities of the isolated compounds are also reported in this paper.

The culture broth extract of BCC 14080 was subjected to Sephadex LH-20 and silica gel column chromatography to afford compound **4** and a subfraction that was subsequently purified by reversed-phase HPLC column chromatography to furnish compound **1**. The crude mycelial extract was separated on a Sephadex LH-20 column to yield compounds **2** and **3**.

Compound **1** was isolated as a white solid. The molecular formula $C_{33}H_{48}O_{10}$ was deduced from HRMS (ESITOF). The IR spectrum showed absorption bands at 3423 (OH) and 1728 (ester carbonyl) cm^{-1}. ^1H NMR, ^{13}C NMR, and 2D NMR data (COSY, NOESY, HMQC, and HMBC) revealed that **1** possessed a sugar unit attached to a 3-heptyl-5-hydroxyphenyl 2-heptyl-4,6-dihydroxybenzoate (KS-501a).[11] Analysis of ^{13}C NMR and DEPT spectra indicated the presence of an ester carbonyl (δ_C 166.4), four oxygenated aromatic carbons (δ_C 159.8, 158.2, 157.2, 152.1), three aromatic quaternary carbons (δ_C 145.2, 143.3, 115.5), five aromatic methines (δ_C 112.8, 112.7, 110.4, 106.8, 101.5), 12 methylenes, and two methyl groups for the aglycone unit. The ^1H and ^{13}C NMR data of the aglycone were close to those of KS-501a reported in the literature.[11] For the sugar unit, an anomeric carbon (δ_C 103.0), four oxymethine carbons (δ_C 75.7, 73.8, 71.4, 69.0), and an oxymethylene carbon (δ_C 61.5) were observed from ^{13}C NMR and DEPT spectra. The vicinal coupling constants, $J_{1'',2''} = 7.7$ Hz, $J_{2'',3''} = 9.4$ Hz, and $J_{3'',4''} = 3.3$ Hz, suggested that H-1″, H-2″, and H-3″ were axial in orientation, while H-4″ was equatorial in the pyranose ring. NOESY cross signals observed from H-1″ to H-3″ and from H-1″ to H-5″ indicated that the sugar unit of **1** was β-galactopyranose. Attachment of the sugar unit to C-2 of the aglycone was established on the basis of HMBC correlation from H-1″ (δ_H 4.86) to C-2 (δ_C 157.2). The ^1H NMR spectrum (in D_2O) and the specific rotation ($[\alpha]^{25}_D$ +71.6, *c* 0.05, H_2O, 24 h) of the aqueous layer of the hydrolysate were identical to those of known



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D-galactose. The ^1H NMR and MS data of the organic layer of the hydrolysate were identical to 5-heptylresorcinol.[12] Two molar equivalents of 5-heptylresorcinol were obtained instead of an equimolar mixture of 5-heptylresorcinol and 2-heptyl-4,6-dihydroxybenzoic acid, since thermal decarboxylation of 2-heptyl-4,6-dihydroxybenzoic acid occurred to yield 5-heptylresorcinol.[11,13] This result also confirmed that both alkyl chains attached to C-6 and C-5′ were the same (*n*-heptyl group).

The molecular formula of **2** was established as $C_{39}H_{58}O_{15}$ on the basis of HRESIMS. The ^1H and ^{13}C NMR data of the aglycone of **2** were nearly identical to those of **1**. For the sugar part, two anomeric carbons (δ_C 103.9, 102.1), eight oxymethine carbons, and two oxymethylene carbons (δ_C 68.6, 61.2) were observed in ^{13}C NMR and DEPT spectra. Analysis of 2D NMR data indicated that the disaccharide unit was composed of two β-galactopyranose moieties. The connectivity between the two sugar units was determined on the basis of HMBC correlations from H-1‴ (δ_H 4.43) to C-6″ (δ_C 68.6) and from H-6″ (δ_H 3.97 and 3.93) to C-1‴ (δ_C 103.9). The (1→6)-linkage was confirmed by the ^1H NMR spectrum (in DMSO-d_6) that revealed six doublet signals and only one triplet signal due to OH protons. HMBC correlation from H-1″ (δ_H 4.89)

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Table 1. Biological Activities of Compounds **1**−**4**[a]

compound	antimalaria, IC_{50} (μM)	anti-HSV-1, IC_{50} (μM)	cytotoxicity, IC_{50} (μM)			
			KB cells	BC cells	NCI-H187 cells	Vero cells
1	>10	7.2 ± 2.1	>25	4.4 ± 1.3	13.9 ± 1.6	43.2 ± 4.3
2	>10	>50	>25	20.2 ± 2.5	>25	32.1 ± 3.6
3 (KS-501a)	9.9 ± 0.9	>100	13.0 ± 1.2	8.8 ± 1.8	13.6 ± 1.9	34.3 ± 1.3
4	>25	>100	>50	>50	>50	>100
dihydroartemisinin[b]	0.0039 ± 0.0003					
acyclovir[c]		10.2 ± 3.6				
ellipticine[d]			1.99 ± 0.23	0.49 ± 0.12	1.71 ± 0.19	1.94 ± 0.13

[a] The IC_{50} values are given as the mean ± SD (n = 3). [b] Standard antimalarial compound. [c] Standard compound for antiherpes simplex virus type 1 (HSV-1) assay. [d] Standard compound for cytotoxicity assays against oral human epidermal carcinoma (KB), human breast cancer (BC), human small-cell lung cancer (NCI-H187), and African green monkey kidney fibroblast (Vero) cells.

to C-2 (δ_C 156.8) indicated attachment of the digalactopyranose moiety to C-2 of the aglycone. Analysis of the acid hydrolysate of **2** yielded D-galactopyranose as the only sugar. The methanolysis of **2** also yielded its aglycone (**3**), which was spectroscopically identical to KS-501a[11] (51%), along with 5-heptylresorcinol[12] (31%) and methyl 2-heptyl-4,6-dihydroxybenzoate[14] (34%).

Compound **3**, the aglycone of compounds **1** and **2**, was also isolated as cometabolite from BCC 14080. Its spectroscopic data were identical to those of KS-501a.[11] KS-501a was previously described as the synthetic product from methanolysis of its β-D-galactofuranoside (KS-501), which was the secondary metabolite of the fungus *Sporothrix* sp. KAC-1985.[11] Herein, KS-501a is reported as a naturally occurring substance.

Compound **4** has the molecular formula $C_{20}H_{32}O_7$ as established by HRMS (ESITOF). It consisted of a sugar unit connected to a 5-heptylresorcinol.[12] The sugar unit of **4** was different from that found in **1** and **2**. The vicinal coupling constants of H-1′ to H-5′ ($J_{1',2'}$ = 7.8 Hz, $J_{2',3'}$ = 9.0 Hz, $J_{3',4'}$ = 9.0 Hz, $J_{4',5'}$ = 9.0 Hz) confirmed that H-1′ to H-5′ were all oriented in the axial positions of a pyranose ring. HMBC correlations observed from methoxy protons (δ_H 3.54; 3H, s) to C-4′ (δ_C 79.4) and from H-4′ (δ_H 3.17; 1H, t, J = 9.0 Hz) to a methoxy carbon (δ_C 59.7, 4′-OCH_3) indicated that the sugar unit was 4-*O*-methyl-β-glucopyranose. The connectivity of this sugar unit to aglycone was deduced by HMBC correlation from H-1′ (δ_H 4.83) to C-1 (δ_C 158.9). Acid hydrolysis of **4** was not performed due to its small quantity; therefore, the configuration of 4-*O*-methylglucopyranose was not determined.

All isolated compounds were screened for antimalarial, cytotoxic, and anti-HSV-1 activities. Compound **1** displayed anti-HSV-1 activity with an IC_{50} value of 7.2 μM. Its aglycone, compound **3**, was active against malaria parasite *Plasmodium falciparum* K1 with an IC_{50} value of 9.9 μM. Compounds **1**−**3** were cytotoxic against BC cell lines with IC_{50} values of 4.4, 20.2, and 8.8 μM, respectively. Cytotoxic activities against KB, NCI-H187, and nonmalignant Vero cell lines are shown in Table 1. The phenolic glycoside (**4**) was inactive against *Plasmodium falciparum* K1 (at 25 μM) and noncytotoxic against three cancer cell lines (at 50 μM).

Experimental Section

General Experimental Procedures. Melting points were measured on an Electrothermal IA9100 digital melting point apparatus. Optical rotations were conducted by using a JASCO P-1030 digital polarimeter. UV and IR spectra were recorded on a Varian Cary 1E UV–vis spectrophotometer and a Bruker VECTOR 22 spectrometer, respectively. NMR spectra were taken on a Bruker AV500D spectrometer. ESITOF mass spectra were recorded on a Micromass LCT spectrometer.

Fungal Material. The fungus *Acremonium* sp. was collected on a palm leaf from Khao Yai National Park, Nakhon Ratchasima Province, and isolated by Mr. Prasert Srikitikulchai. This fungus was deposited at the BIOTEC Culture Collection as BCC 14080 on September 12, 2003. Genomic DNA of BCC 14080 was extracted from lyophilized mycelia (50–100 mg) by CTAB method.[15] The ITS1-5.8S-ITS2 region of rDNA sequences was amplified using the universal primers ITS4 and ITS5.[16] PCR amplification was carried out following the condition described previously.[17] PCR products were purified with a QIAquick PCR purification kit (QIAGEN) and sequenced by Macrogen Inc., Korea. Phylogenetic analysis of the sequence data using BioEdit 7.0.5 and PAUP programs combined with morphological data indicated that BCC 14080 belongs to the genus *Acremonium*. The fungus sequence was submitted to GenBank with accession number EU488735.

Fermentation, Extraction, and Isolation. The fungus BCC 14080 was cultured in 10 L (40 × 250 mL) of bacto-malt extract broth on rotary shakers (200 rpm) for 32 days at 25 °C, and then the culture was filtered. The filtrate was extracted twice with an equal volume of EtOAc. The organic layer was concentrated under reduced pressure to obtain a dark brown gum (2.12 g). The broth extract was passed through a Sephadex LH-20 column (elution with 100% MeOH) to afford eight fractions (1–8). Fraction 4 yielded pure compound **2** (1.31 g). Fraction 6 was subjected to silica gel column chromatography (step gradient elution with 0–15% MeOH/$CHCl_3$) to provide 14 subfractions (6-1–6-14). Fraction 6-9 was pure compound **4** (4.7 mg). Compound **1** (9.2 mg) was obtained from fraction 6-11 after further purification by preparative HPLC using a reversed-phase column (Nova-Pak HR C_{18}, 6 μm, 25 × 100 mm; MeOH/H_2O, 85:15).

The mycelial cakes were macerated with MeOH (1 L, 2 days) and filtered. To the filtrate was added H_2O (100 mL), and the mixture was washed with hexane (700 mL). The aqueous MeOH layer was concentrated under reduced pressure. The residue was dissolved in EtOAc (700 mL) and washed with H_2O (200 mL). The organic layer was concentrated under reduced pressure to leave a pale yellow solid (3.65 g). This mycelial extract was subjected to Sephadex LH-20 column chromatography (elution with 100% MeOH) to yield compounds **2** (429 mg), and **3** (8.5 mg).

Compound 1: white solid; mp 174–176 °C; $[\alpha]^{27}_D$ –3.3 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 203 (4.66), 255 (3.87) nm; IR ($CHCl_3$) ν_{max} 3423, 2926, 1728, 1613, 1592, 1465, 1260, 1073 cm^{-1}; ^1H NMR (acetone-d_6, 500 MHz) δ 6.70 (1H, t, J = 2.1 Hz, H-2′), 6.68 (1H, d, J = 2.1 Hz, H-3), 6.59 (2H, br m, H-4′ and H-6′), 6.49 (1H, d, J = 2.1 Hz, H-5), 4.86 (1H, d, J = 7.7 Hz, H-1″), 3.96 (1H, br d, J = 3.3 Hz, H-4″), 3.84 (1H, dd, J = 9.4, 7.7 Hz, H-2″), 3.83 (1H, dd, J = 11.2, 6.2 Hz, H-6″a), 3.78 (1H, dd, J = 11.2, 5.1 Hz, H-6″b), 3.75 (1H, br dd, J = 6.2, 5.1 Hz, H-5″), 3.64 (1H, dd, J = 9.4, 3.3 Hz, H-3″), 2.72 (1H, dt, J = 13.4, 8.0 Hz, H-7a), 2.65 (1H, dt, J = 13.4, 8.0 Hz, H-7b), 2.56 (2H, t, J = 7.9 Hz, H-7′), 1.62 (4H, m, H-8 and H-8′), 1.39−1.22 (16H, m, H-9−H-12 and H-9′−H-12′), 0.87 (3H, t, J = 7.0 Hz, H-13 or H-13′), 0.86 (3H, t, J = 6.90 Hz, H-13′ or H-13); ^{13}C NMR (acetone-d_6, 125 MHz) δ 166.4 (C, C=O), 159.8 (C, C-4), 158.2 (C, C-3′), 157.2 (C, C-2), 152.1 (C, C-1′), 145.2 (C, C-5′), 143.3 (C, C-6), 115.5 (C, C-1), 112.8 (CH, C-4′ or C-6′), 112.7 (CH, C-6′ or C-4′), 110.4 (CH, C-5), 106.8 (CH, C-2′), 103.0 (CH, C-1″), 101.5 (CH, C-3), 75.7 (CH, C-5″), 73.8 (CH, C-3″), 71.4 (CH, C-2″), 69.0 (CH, C-4″), 61.5 (CH_2, C-6″), 35.5 (CH_2, C-7′), 33.6 (CH_2, C-7), 31.7 (CH_2, C-11 and C-11′), 31.4 (CH_2, C-8′ or C-8), 31.1 (CH_2, C-8 or C-8′), 29.4 and 29.0 (CH_2, C-9, C-10, C-9′, and C-10′), 22.4 (CH_2, C-12 and C-12′), 13.5 (CH_3, C-13 and C-13′); HRMS (ESITOF) *m*/*z* 627.3152 $[M + Na]^+$ (calcd for $C_{33}H_{48}O_{10}Na$, 627.3145).

Compound 2: pale yellow solid; mp 200–202 °C; $[\alpha]^{26}_D$ –10.6 (*c* 0.20, MeOH); UV (MeOH) λ_{max} (log ε) 203 (5.10), 253 (4.26) nm; IR ($CHCl_3$) ν_{max} 3406, 2926, 1725, 1620, 1590, 1465, 1259, 1076 cm^{-1}; ^1H NMR (acetone-d_6/D_2O, 95:5, 500 MHz) δ 6.92 (1H,

d, J = 2.1 Hz, H-3), 6.65 (1H, t, J = 2.1 Hz, H-2′), 6.58 (1H, br m, H-4′), 6.52 (1H, br m, H-6′), 6.43 (1H, d, J = 2.1 Hz, H-5), 4.89 (1H, d, J = 7.7 Hz, H-1″), 4.43 (1H, d, J = 7.7 Hz, H-1‴), 4.05 (1H, br dd, J = 7.0, 5.2 Hz, H-5″), 3.97 (1H, dd, J = 11.7, 7.0 Hz, H-6″a), 3.94 (1H, br d, J = 3.5 Hz, H-4″), 3.93 (1H, dd, J = 11.7, 5.2 Hz, H-6″b), 3.88 (1H, br d, J = 3.4 Hz, H-4‴), 3.82 (1H, dd, J = 9.5, 7.7 Hz, H-2″), 3.77 (1H, dd, J = 11.2, 6.9 Hz, H-6‴a), 3.72 (1H, dd, J = 11.2, 5.2 Hz, H-6‴b), 3.65 (1H, dd, J = 9.5, 3.5 Hz, H-3″), 3.59 (1H, dd, J = 9.7, 7.7 Hz, H-2‴), 3.55 (1H, br dd, J = 6.9, 5.2 Hz, H-5‴), 3.52 (1H, dd, J = 9.7, 3.4 Hz, H-3‴), 2.66 (2H, m, H-7), 2.54 (2H, t, J = 8 Hz, H-7′), 1.62 (2H, m, H-8), 1.60 (2H, m, H-8′), 1.40−1.22 (16H, m, H-9−H-12 and H-9′−H-12′), 0.85 (6H, t, J = 7.0 Hz, H-13 and H-13′); ^{1}H NMR (DMSO-d_6, 500 MHz,) δ 9.73 (1H, s, 4-OH), 9.56 (1H, s, 3′-OH), 6.65 (1H, d, J = 1.7 Hz, H-3), 6.51 (1H, br s, H-4′), 6.48 (1H, t, J = 1.9 Hz, H-2′), 6.43 (1H, br s, H-6′), 6.34 (1H, d, J = 1.7 Hz, H-5), 5.13 (1H, d, J = 4.6 Hz, 2‴-OH), 4.93 (1H, d, J = 5.9 Hz, 3″-OH), 4.81 (1H, d, J = 7.8 Hz, H-1″), 4.80 (1H, d, J = 5.6 Hz, 2″-OH), 4.75 (1H, d, J = 5.6 Hz, 3‴-OH), 4.65 (1H, d, J = 4.8 Hz, 4″-OH), 4.60 (1H, t, J = 5.6 Hz, 6‴-OH), 4.51 (1H, d, J = 4.7 Hz, 4‴-OH), 4.19 (1H, d, J = 7.6 Hz, H-1‴), 3.84 (2H, m, H-5″ and H-6″a), 3.74 (1H, t, J = 4.0 Hz, H-4″), 3.67 (1H, dd, J = 12.2, 8.2 Hz, H-6″b), 3.62 (1H, t, J = 3.8 Hz, H-4‴), 3.57 (1H, ddd, J = 9.2, 7.8, 5.6 Hz, H-2″), 3.51 (1H, ddd, J = 11.0, 5.6, 5.1 Hz, H-6‴a), 3.48 (1H, ddd, J = 11.0, 5.6, 5.2 Hz, H-6‴b), 3.42 (1H, ddd, J = 9.2, 5.9, 4.0 Hz, H-3″), 3.34 (2H, m, H-2‴ and H-5‴), 3.26 (1H, ddd, J = 9.3, 5.6, 3.8 Hz, H-3‴), 2.57 (2H, m, H-7), 2.50 (2H, m, H-7′), 1.53 (4H, m, H-8 and H-8′), 1.28−1.23 (16H, m, H-9–H-12 and H-9′–H-12′), 0.85 (3H, t, J = 6.8 Hz, H-13 or H-13′), 0.84 (3H, t, J = 7.3 Hz, H-13′ or H-13); ^{13}C NMR (acetone-d_6/D_2O, 95:5, 125 MHz) δ 166.6 (C, C═O), 159.8 (C, C-4), 158.2 (C, C-3′), 156.8 (C, C-2), 152.0 (C, C-1′), 145.1 (C, C-5′), 143.1 (C, C-6), 114.6 (C, C-1), 112.9 (CH, C-4′), 112.4 (CH, C-6′), 110.2 (CH, C-5), 106.7 (CH, C-2′), 103.9 (CH, C-1‴), 102.1 (CH, C-1″), 101.1 (CH, C-3), 75.2 (CH, C-5‴ or C-5″), 74.9 (CH, C-5″ or C-5‴), 73.4 (CH, C-3″ or C-3‴), 73.3 (CH, C-3‴ or C-3″), 71.7 (CH, C-2‴), 71.3 (CH, C-2″), 68.9 (CH, C-4‴ or C-4″), 68.7 (CH, C-4″ or C-4‴), 68.6 (CH_2, C-6″), 61.2 (CH_2, C-6‴), 35.5 (CH_2, C-7′), 33.6 (CH_2, C-7), 31.7 (CH_2, C-11 or C-11′), 31.6 (CH_2, C-11′ or C-11), 31.4 (CH_2, C-8′), 31.1 (CH_2, C-8), 29.4 and 29.0 (CH_2, C-9, C-10, C-9′, and C-10′), 22.4 (CH_2, C-12 and C-12′), 13.5 (CH_3, C-13 and C-13′); HRMS (ESITOF) m/z 789.3688 $[M + Na]^+$ (calcd for $C_{39}H_{58}O_{15}Na$, 789.3673).

Compound 4: pale yellow solid; mp 132–134 °C; $[\alpha]^{26}_D$ –47.3 (c 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 203 (4.86), 219 (4.35), 269 (4.01) nm; IR ($CHCl_3$) ν_{max} 3384, 2926, 1622, 1594, 1465, 1309, 1178, 1093, 1057 cm^{-1}; ^{1}H NMR (acetone-d_6/D_2O, 95:5, 500 MHz,) δ 6.39 (1H, t, J = 2.2 Hz, H-2), 6.35 (1H, br d, J = 2.2 Hz, H-6), 6.32 (1H, br d, J = 2.2 Hz, H-4), 4.83 (1H, d, J = 7.8 Hz, H-1′), 3.81 (1H, dd, J = 12.0, 2.1 Hz, H-6′a), 3.66 (1H, dd, J = 12.0, 5.1 Hz, H-6′b), 3.59 (1H, t, J = 9.0 Hz, H-3′), 3.54 (3H, s, 4′-OCH_3), 3.42 (1H, ddd, J = 9.0, 5.1, 2.1 Hz, H-5′), 3.41 (1H, dd, J = 9.0, 7.8 Hz, H-2′), 3.17 (1H, t, J = 9.0 Hz, H-4′), 2.45 (2H, t, J = 7.5 Hz, H-7), 1.54 (2H, quin, J = 7.5 Hz, H-8), 1.22–1.35 (8H, m, H-9–H-12), 0.85 (3H, t, J = 7.0 Hz, H-13); ^{13}C NMR (acetone-d_6/D_2O, 95:5, 125 MHz) δ 158.9 (C, C-1), 158.3 (C, C-3), 144.9 (C, C-5), 109.2 (CH, C-4), 107.7 (CH, C-6), 101.1 (CH, C-2), 100.7 (CH, C-1′), 79.4 (CH, C-4′), 76.9 (CH, C-3′), 75.9 (CH, C-5′), 73.9 (CH, C-2′), 61.1 (CH_2, C-6′), 59.7 (CH_3, 4′-OCH_3), 35.7 (CH_2, C-7), 31.7 (CH_2, C-11), 31.1 (CH_2, C-8), 29.0 (CH_2, C-9 and C-10), 22.4 (CH_2, C-12), 13.4 (CH_3, C-13); HRMS (ESITOF) m/z 407.2044 $[M + Na]^+$ (calcd for $C_{20}H_{32}O_7Na$, 407.2046).

Hydrolysis of 1. Compound **1** (4.0 mg) was hydrolyzed with 5% HCl (1 mL) at 90 °C for 12 h. The reaction mixture was then diluted with H_2O (3 mL) and extracted with EtOAc (3 × 5 mL). The aqueous layer was concentrated *in vacuo* to yield an anomeric mixture of D-galactose (0.98 mg; $[\alpha]^{25}_D$ +71.6, c 0.05, H_2O, 24 h). The specific rotation, $[\alpha]^{25}_D$ +79.0 (c 1.0, H_2O, 24 h), was observed for an anomeric mixture of D-galactose standard. The organic layer was evaporated to dryness and then purified by a Sephadex LH-20 column (elution with 100% MeOH) to obtain pure compound (2.5 mg), whose MS and ^{1}H NMR spectra were identical to the literature data of 5-heptylresorcinol.[12]

Hydrolysis of 2. Hydrolysis of compound **2** (65.0 mg) was performed in the same manner as **1**. The aqueous layer was concentrated *in vacuo* to yield an anomeric mixture of D-galactose (23.7 mg; $[\alpha]^{27}_D$ +89.6, c 1.2, H_2O, 24 h). The organic layer was concentrated and purified by a Sephadex LH-20 column (elution with 100% MeOH) to obtain 5-heptylresorcinol[12] (24.0 mg).

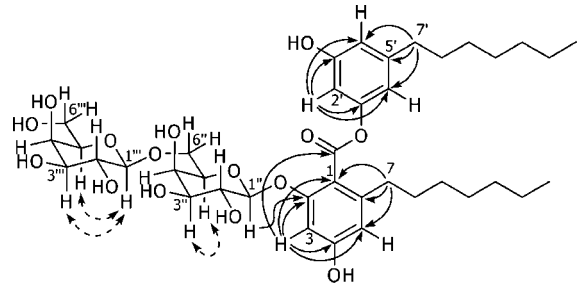

Figure 1. Selected NOESY (dashed lines) and HMBC (solid lines) correlations for compound **2**.

Methanolysis of 2. Anhydrous HCl gas was bubbled through MeOH (15 mL) for 15 min. Compound **2** (200.0 mg) was dissolved in HCl/MeOH (5 mL) and then heated to 70 °C for 12 h. The reaction mixture was diluted with H_2O (10 mL) and extracted with EtOAc (3 × 15 mL). The organic layer was evaporated to dryness, and the residue was purified by silica gel column chromatography (step gradient elution with 0–20% MeOH/$CHCl_3$) to obtain compound **3** (59.4 mg), 5-heptylresorcinol[12] (17.0 mg), and methyl 2-heptyl-4,6-dihydroxybenzoate[14] (23.4 mg).

Biological Assays. Antimalarial activity against *Plasmodium falciparum* K1 was evaluated by using microculture radioisotope technique.[18] Antiherpes simplex virus type 1 (HSV-1) assay and cytotoxicity assays against oral human epidermal carcinoma (KB) cells, human breast cancer (BC) cells, human small-cell lung cancer (NCI-H187) cells, and African green monkey kidney fibroblast (Vero) cells were carried out by employing a colorimetric method.[19,20]

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References and Notes

(1) Evans, L.; Hedger, J. N.; Brayford, D.; Stavri, M.; Smith, E.; O'Donnell, G.; Gray, A. I.; Griffith, G. W.; Gibbons, S. *Phytochemistry* **2006**, *67*, 2110–2114.
(2) Strobel, G. A.; Torczynski, R.; Bollon, A. *Plant Sci.* **1997**, *128*, 97–108.
(3) Ahn, J. S.; Kim, B. Y.; Oh, W. K.; Mheen, T. I.; Ahn, S. C.; Kang, D. O.; Ko, H. R.; Kim, H. M. WO 0146385, 2001.
(4) Aoki, K.; Uchiyama, R.; Itonori, S.; Sugita, M.; Che, F.-S.; Isogai, A.; Hada, N.; Hada, J.; Takeda, T.; Kumagai, H.; Yamamoto, K. *Biochem. J.* **2004**, *378*, 461–472.
(5) Chen, C.; Imamura, N.; Nishijima, M.; Adachi, K.; Sakai, M.; Sano, H. *J. Antibiot.* **1996**, *49*, 998–1005.
(6) Boot, C. M.; Amagata, T.; Tenney, K.; Compton, J. E.; Pietraszkiewicz, H.; Valeriote, F. A.; Crews, P. *Tetrahedron* **2007**, *63*, 9903–9914.
(7) Nakai, R.; Ogawa, H.; Asai, A.; Ando, K.; Agatsuma, T.; Matsumiya, S.; Akinaga, S.; Yamashita, Y.; Mizukami, T. *J. Antibiot.* **2000**, *53*, 294–296.
(8) Kleinwachter, P.; Schlegel, B.; Dorfelt, H.; Grafe, U. *J. Antibiot.* **2001**, *54*, 526–527.
(9) Sassa, T.; Ooi, T.; Kinoshita, H. *Biosci. Biotechnol. Biochem.* **2004**, *68*, 2633–2636.
(10) (a) Bunyapaiboonsri, T.; Yoiprommarat, S.; Intereya, K.; Kocharin, K. *Chem. Pharm. Bull.* **2007**, *55*, 304–307. (b) Isaka, M.; Kittakoop, P.; Kirtikara, K.; Hywel-Jones, N. L.; Thebtaranonth, Y. *Acc. Chem. Res.* **2005**, *38*, 813–823.
(11) (a) Yasuzawa, T.; Saitoh, Y.; Sano, H. *J. Antibiot.* **1990**, *43*, 336–343. (b) Nakanishi, S.; Toki, S.; Kase, H.; Matsuda, Y.; Shiozaki, S.; Shuto, K.; Ikeda, J.; Kubo, K. Jpn. Kokai Tokkyo Koho JP 02256619, 1990.
(12) Arisawa, M.; Ohmura, K.; Kobayashi, A.; Morita, N. *Chem. Pharm. Bull.* **1989**, *37*, 2431–2434.
(13) (a) Anker, R. M.; Cook, A. H. *J. Chem. Soc.* **1945**, 311–313. (b) Huneck, S.; Schmidt, J.; Tabacchi, R. Z. *Naturforsch., B: Chem. Sci.* **1989**, *44*, 1283–1289.

(14) Djura, P.; Sargent, M. V. *Aust. J. Chem.* **1976**, *29*, 899–906.
(15) White, T. J.; Bruns, T. D.; Lee, S.; Taylor, J. W. In *PCR Protocols: A Guide to Methods and Applications*; Innis, M. A.; Gelfand, D. H.; Sninsky, J. J.; White, T. J., Eds.; Academic Press: San Diego, 1990; pp 315–322.
(16) Luangsa-ard, J. J.; Hywel-Jones, N. L.; Manoch, L.; Samson, R. A. *Mycol. Res.* **2005**, *109*, 581–589.
(17) O'Donnell, K.; Cigelnik, E.; Weber, N. S.; Trappe, J. M. *Mycologia* **1997**, *89*, 48–65.
(18) Desjardins, R. E.; Canfield, C. J.; Haynes, J. D.; Chulay, J. D. *Antimicrob. Agents Chemother.* **1979**, *16*, 710–718.
(19) Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. *J. Natl. Cancer Inst.* **1990**, *82*, 1107–1112.
(20) Plumb, J. A.; Milroy, R.; Kaye, S. B. *Cancer Res.* **1989**, *49*, 4435–4440.

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