Aurocitrin and Related Polyketide Metabolites from the Wood-Decay Fungus *Hypocrea* sp. BCC 14122

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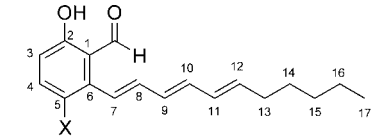
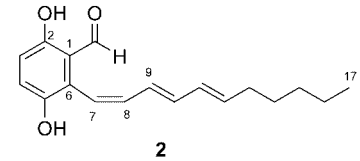
The known gentisaldehyde antibiotic aurocitrin (**1**), its *cis*-olefinic isomer (**2**), a salicylaldehyde analogue (**3**), two new benzofuran derivatives (**4** and **5**), and a new dihydroisocoumarin (**6**) were isolated from the wood-decay fungus *Hypocrea* sp. BCC 14122. The structures were elucidated primarily by NMR and mass spectroscopic analyses.

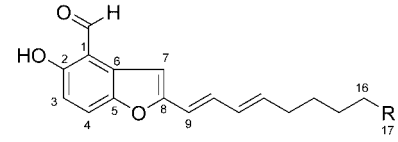
As a part of our continuing search for biologically active compounds from bioresources in Thailand,[1−3] we have investigated the constituents of a wood-decay fungus, *Hypocrea* sp. BCC 14122, because an extract of this strain had shown activity against human small cell lung cancer cells (NCI-H187, IC_{50} 5.2 μg/mL). Chemical studies led to the identification of a known gentisaldehyde derivative, aurocitrin (**1**),[4] as the most abundant metabolite, and five related new compounds **2**−**6** as minor constituents. Details of the isolation, structure elucidation, and biological activities are presented here. Aurocitrin (**1**) was previously isolated from *Hypocrea citrina*.[4,5] There have been a few other reports on the secondary metabolites of *Hypocrea* species, such as the trichothecene harzianum A from *Hypocrea* sp. F000527[6] and *H. lutea*[7] and peptibol-type polypeptides from *H. peltata*.[8,9] However, many species in the genus *Hypocrea* are teleomorphs (sexual state) of *Trichoderma* species, which are rich sources of bioactive compounds.[10]

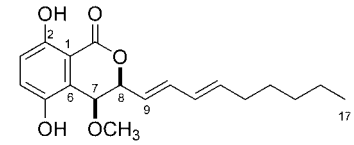
Aurocitrin (**1**) was identified on the basis of the NMR and MS data. The conjugated triene moiety with all-*trans*-geometry (7*E*,9*E*,11*E*) was assigned from the vicinal $^1H-^1H$ *J* values. The physicochemical data of **1** were identical in all respects to those reported in the literature.[4]

Compound **2** possesses the same molecular formula as **1**, $C_{18}H_{22}O_3$ (ESIMS, $\Delta = 1.8$ ppm for $[M + Na]^+$). The ^1H and ^{13}C NMR spectra were similar to those of **1** except for the ^1H chemical shifts of the olefinic protons. The vicinal coupling constant of $J_{7,8} = 11.0$ Hz clearly indicated the *cis*-geometry of this olefinic bond, whereas the other double bonds were *trans* ($J_{9,10} = 14.9$ Hz, $J_{11,12} = 15.1$ Hz). The structure of the gentisaldehyde moiety was evident from the appearance of a formyl group (δ_H 10.03, s; δ_C 196.3), two vicinally coupled aromatic protons (δ_H 6.88 and 7.18; $J_{3,4} =$ 8.9 Hz), and two phenolic protons, one of which was chelated (δ_H 11.41, s, 2-O*H*). HMBC correlation data were consistent with the proposed aromatic moiety: formyl proton to C-1 and C-2, chelated OH proton (2-OH) to C-1, C-2, and C-3, H-3 to C-1 and C-5, and H-4 to C-2 and C-6. The HMBC correlation from an olefinic proton (H-7, δ_H 6.33) to C-5 (δ_C 145.1) indicated that the triene terminal (C-7) of the linear side chain (C-7−C-17) was attached to C-6 of the benzene ring. Therefore, compound **2** was assigned as (7*Z*,9*E*,11*E*)-aurocitrin, a new *cis*-olefin analogue of **1**. It should be noted that compound **2** was isolated with trace contamination of **1** (2–3%), as indicated by the ^1H NMR spectrum; however, the composition of **1** increased to ca. 15% when the sample was recovered and the ^1H NMR spectrum taken again after 2 weeks. This observation demonstrated the ease of olefin isomerization from 7*Z* to more stable









7*E*. On the other hand, we observed no evidence of isomerization from 7*E* to 7*Z* during the isolation of **1**, **2**, and **3**.

The molecular formula of compound **3** was determined by HRMS (ESITOF) as $C_{18}H_{22}O_2$, one less oxygen atom than **1**. The ^1H and ^{13}C NMR spectra of **3** were similar to those of **1** for the linear chain portion (C-7−C-17); however, they differed in the aromatic ring. Three aromatic methine signals at δ_H 6.84 (d, $J = 8.3$ Hz), 7.44 (t, $J = 8.0$ Hz), and 7.00 (d, $J = 7.7$ Hz) were assigned respectively to H-3, H-4, and H-5. The formyl group was found at δ_H 10.33 (δ_C 195.2), and a chelated OH group resonated as a sharp singlet at δ_H 11.94 (2-O*H*). No other OH group was evident, and these data indicated that **3** was the 5-deoxy analogue of **1**. The salicylaldehyde moiety was confirmed by detailed analysis of the 2D NMR data; especially key HMBC correlations were observed from H-5 to C-7, from H-7 to C-5, and from H-4 and H-8 to C-6. The (7*E*,9*E*,11*E*)-geometry of the conjugated triene system was established from the $^1H-^1H$ *J* values of 15.2, 14.9, and 15.1 Hz.

The molecular formula of compound **4** was determined to be $C_{18}H_{20}O_3$ by HRMS (ESITOF), two hydrogen atoms less than **1**. The ^1H and ^{13}C NMR spectra suggested that this compound was

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also related to **1**; however, one of the double bonds in the conjugated triene system had an oxygen attached, and the ^{1}H and ^{13}C chemical shifts of the aromatic ring moiety differed slightly from those of **1**. Instead of the C-7−C-8 *trans*-olefin in **1**, resonances of a downfield quaternary sp^2 carbon (δ_C 158.9) and an sp^2 methine (δ_H 6.84, br s; δ_C 100.0) were present. The conjugated *trans*−*trans* diene, C-9−C-12, possessed a (9*E*,11*E*)-configuration on the basis of the coupling constant values of $J_{9,10}$ = 15.5 Hz and $J_{11,12}$ = 15.0 Hz. One side of this diene was attached to an *n*-pentyl group (C-13−C-17). The other side (C-9; H-9, δ_H 6.35, d, J = 15.5 Hz) was connected to a δ_C 158.9 quaternary carbon (C-8). The C-8 assignment was based on the HMBC correlations from H-9 and H-10 to this carbon. The δ_H 6.84 sp^2 methine proton (H-7) showed HMBC correlations to C-6 and C-8. On the basis of these NMR data, and taken together with the molecular formula (HRMS), a benzofuran unit was proposed for **4**. The structure of this aromatic portion was confirmed by HMBC correlations: from the formyl proton (δ_H 10.27, s) to C-1 and C-2, from the chelated OH (2-O*H*, δ_H 11.37, s) to C-1, C-2, and C-3, from H-3 to C-1, C-2, and C-5, and from H-4 to C-2 and C-6. Compound **4** may be derived from aurocitrin (**1**) through oxidation of the C-7−C-8 olefin to give a 7,8-dihydroxy, 7,8-epoxy, or 8-keto derivative, followed by formation of the benzofuran.

The ^{1}H NMR and ^{13}C NMR spectra of compound **5** were very similar to those of **4**; however, significant differences were the absence of the terminal methyl group and the appearance of a carboxyl ^{13}C resonance at δ_C 177.0 in **5**. The molecular formula of **5** was established as $C_{18}H_{18}O_5$ by HRMS, consistent with the structure in which the terminal methyl (C-17) in **4** was oxygenated to a carboxyl group in **5**. One of the methylene resonances was relatively downfield shifted and appeared as a triplet signal (2H, δ_H 2.39, t, J = 7.4 Hz). Analysis of COSY and HMQC spectra revealed the assignments and connection of the *trans*−*trans* diene and four methylenes, C-9 to C-16. HMBC correlations from H-15 and H-16 to δ_C 177.0 (C-17) confirmed the terminal carboxylic acid functionality. The structure of the benzofuran moiety was elucidated by analysis of 2D NMR data, especially HMBC correlations, which provided the same correlation data as found for **4**.

A minor constituent (**6**, 0.2 mg) was also isolated. The molecular formula, $C_{19}H_{24}O_4$ (HRMS and NMR), suggested that this compound was also structurally related to aurocitrin (**1**). The most significant differences were the absence of a formyl group and the appearance of carboxy (δ_C 168.4) and methoxy (δ_H 3.48, 3H, s; δ_C 57.1) signals. In addition, the C-7−C-8 *trans*-olefin was replaced by two oxymethines that resonated at δ_H 4.95 (d, J = 3.6 Hz, H-7; δ_C 72.9) and 5.08 (dd, J = 7.6, 3.6 Hz, H-8; δ_C 80.2). The COSY data indicated the connection from C-7 to C-17. The location of the methoxy group was indicated by the HMBC correlation from the protons to C-7. The connection of C-7 with C-6 was demonstrated by the HMBC correlation from H-8 to C-6. The substructure of the aromatic moiety was also established on the basis of HMBC correlations: from 2-O*H* (δ_H 10.61, s) to C-1, C-2, and C-3, from H-3 to C-1 and C-5, and from H-4 to C-2, C-5, and C-6. Although the HMBC cross-peak of H-7 with the δ_C 177.0 carbonyl carbon was not observed, the molecular formula (established by HRMS) required the formation of a lactone ring. The downfield shift of H-8 (δ_H 5.08; δ_C 80.2) was consistent with the dihydroisocoumarin skeleton. The small $J_{6,7}$ value (3.6 Hz) indicated pseudoequatorial orientation of H-7, hence a *cis*-relation of the C-9−C-17 side chain (pseudoequatorial) and the methoxy group (pseudoaxial). Because of the shortage of material, the absolute configuration of **6** was not determined.

A benzofuran and isocoumarins, structurally related to **4** and **6** but with a shorter alkyl side chain, were recently isolated from the cultured lichen mycobionts of *Pyrenula* species.[11]

Compounds **1**–**3** exhibited moderate antiplasmodial activity and cytotoxicity against three cancer cell lines, KB, BC, and NCI-H187, as well as noncancerous Vero cells (Table 1). Compounds **1**–**3** were inactive in our antituberculous (*Mycobacterium tuberculosis* $H_{37}Ra$) and antifungal (*Candida albicans*) assays. Aurocitrin (**1**) is known to exhibit activity against *Staphylococcus aureus* in serial dilution tests.[4]

Table 1. Antimalarial (Antiplasmodial) and Cytotoxic Activities of Compounds **1**–**3**

compound	IC$_{50}$ (μM) of *P. falciparum* K1	cytotoxicity (IC$_{50}$, μM) KB	BC	NCI-H187	Vero
1	6.3	4.2	2.5	0.87	15
2	7.0	21	8.3	4.4	35
3	-[c]	13	-[c]	5.0	5.8
dihydroartemisinin[a]	0.0045	-[c]	-[c]	-[c]	-[c]
ellipticine[b]	-[c]	2.2	1.2	2.0	2.1

[a] Standard antimalarial drug. [b] Reference compound for the cytotoxicity assay. [c] Not tested.

Experimental Section

General Experimental Procedures. UV–visible spectra were recorded on a Varian CARY 1E spectrophotometer. FT-IR spectra were recorded on a Bruker VECTOR 22 spectrometer. NMR spectra were recorded on a Bruker AV500D spectrometer. ESITOF mass spectra were measured with a Bruker micrOTOF mass spectrometer.

Fungal Material. The fungus used in this study was isolated from an unidentified twig collected from leaf litter in Khao Sabap National Park, Chanthaburi Province, Thailand, by Dr. Nigel L. Hywel-Jones of BIOTEC. This fungus was deposited in the BIOTEC Culture Collection (BCC) on August 27, 2003, as BCC 14122. The specimen formed discrete orange-brown stromas on the decorticated part of a small twig (ca. 10 mm diameter). Each stroma was 3–5 mm in diameter and consisted of ca. 30–40 perithecia. Stromas were made of pseudoparenchymatous cells, and the whole stained red with 3% KOH. The perithecia contained asci that were ca. 60 × 4 μm. Within the ascus the eight ascospores divided into two dimorphic part-spores. Proximal cells rounded off to produce spores of ca. 2 μm diameter The distal cells were elongate and 3 × 2 μm. These ascospores were discharged onto potato dextrose agar. They germinated and grew to form slow-growing (ca. 60 mm in 4 week) colonies. Colonies produced an *Acremonium* anamorph with phialides ca. 10–15 μm long. These formed spore balls containing 5–10 conidia of ca. 5 × 2.5 μm. All of these characteristics indicate a species of *Hypocrea* close to the *Hypocrea citrina* complex of species. The whole genus is in need of taxonomic revision, and further identification of the Thai material must await such a revision.

Fermentation and Isolation. *Hypocrea* sp. BCC 14122 was cultured in a 10 L bioreactor using potato dextrose broth (PDB, 7 L) at 25 °C for 16 days. The culture was filtered to separate broth (filtrate) and mycelium (residue). The filtrate was extracted with EtOAc (3 × 5 L) to give a dark brown gum (2.29 g, extract A). The wet mycelium was macerated in MeOH (600 mL, 2 days) and filtered. To the methanolic solution were added hexane (500 mL) and H_2O (100 mL), and the layers were separated. The hexane layer was concentrated, under reduced pressure, leaving an oil (278 mg, extract B). The aqueous methanol layer was partially concentrated by evaporation, and the residue was extracted with EtOAc (3 × 700 mL). The combined EtOAc solution was concentrated to a brown gum (441 mg, extract C). Extracts A, B, and C were separately subjected to chromatographic fractionation. Extract B (278 mg) was passed through a column on silica gel (2.8 × 25 cm; eluted with EtOAc/hexane) to obtain five fractions: B-1 to B-5. Fraction B-2 (12 mg) was subjected to semipreparative HPLC using a reversed-phase column (LiChroCART, 10 × 250 mm, 10 μm; MeOH/H_2O = 80:20; flow 4 mL/min) to furnish compounds **4** (1.5 mg) and **3** (2.4 mg). Fraction B-3 (107 mg) was fractionated by column on Sephadex LH-20 (3.0 × 55 cm, MeOH) to obtain compounds **1** (98 mg) and **2**. Compound **2** was further purified by semipreparative HPLC (LiChroCART, 10 × 250 mm, 10 μm; MeOH/H_2O = 80:20; flow 4 mL/min) to furnish a pale brown solid (5.6 mg; t_R 20 min). Extract A (2.29 g) was passed through a Sephadex LH-20 column (3.8 × 55 cm, elution with MeOH) to provide seven fractions. Only the last fraction, A-7 (49 mg), showed a unique ^{1}H NMR profile. The material was further fractionated on Sephadex LH-20 (1.3 × 88 cm, MeOH), then subjected to silica gel column chromatography (CC) (1.0 × 16 cm) to furnish compound **5** (1.3 mg). Extract C (441 mg) was passed through a Sephadex LH-20 column (2.4 × 87 cm, MeOH) to obtain eight

fractions. Fraction C-4 (108 mg) was further fractionated by silica gel CC (2.4 × 23 cm; MeOH/CH_2Cl_2), and the second subfraction (3 mg) was further purified using a short silica gel column (0.7 × 15 cm; EtOAc/hexane) and HPLC (MeOH/H_2O = 70:30; flow 4 mL/min) to give compound **6** (0.2 mg, t_R 11 min).

(7*E*,9*E*,11*E*)-Aurocitrin (1): orange-brown solid; mp 114−115 °C; UV–vis (MeOH) λ_{max} (log ε) 263 (4.66), 313 (4.53), 410 (4.23) nm; IR (KBr) ν_{max} 3443, 1659 sh, 1624, 1572, 1470, 1270, 998 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 11.47 (1H, s, 2-O*H*), 10.09 (1H, s, C*H*O), 7.10 (1H, d, J = 9.0 Hz, H-4), 6.80 (1H, d, J = 9.0 Hz, H-3), 6.67 (1H, d, J = 15.8 Hz, H-7), 6.52 (1H, dd, J = 15.8, 9.8 Hz, H-8), 6.37 (1H, dd, J = 14.8, 9.8 Hz, H-10), 6.34 (1H, dd, J = 14.8, 9.8 Hz, H-9), 6.15 (1H, dd, J = 15.1, 9.8 Hz, H-11), 5.87 (1H, dt, J = 15.1, 7.1 Hz, H-12), 5.11 (1H, br s, 5-O*H*), 2.14 (2H, br q, J = 7.1 Hz, H-13), 1.42 (2H, m, H-14), 1.31 (2H, m, H-15), 1.29 (2H, m, H-16), 0.90 (3H. t, J = 7.0 Hz, H-17); ^{13}C NMR (125 MHz, $CDCl_3$) δ 196.0 (d, *C*HO), 157.2 (s, C-2), 145.6 (s, C-5), 140.4 (d, C-8), 138.9 (d, C-12), 136.9 (d, C-10), 129.8 (d, C-11), 129.0 (d, C-9), 127.0 (s, C-6), 125.6 (d, C-4), 120.4 (d, C-7), 117.6 (s, C-1), 117.4 (d, C-3), 33.0 (t, C-13), 31.4, t, C-15), 28.8 (t, C-14), 22.5 (t, C-16), 14.1 (q, C-17); HRMS (ESITOF) m/z 309.1454 $[M + Na]^+$ (calcd for $C_{18}H_{22}O_3Na$, 309.1461).

(7*Z*,9*E*,11*E*)-Aurocitrin (2): pale brown solid; mp 100−101 °C; UV–vis (MeOH) λ_{max} (log ε) 269 (4.59), 392 (3.81) nm; IR (KBr) ν_{max} 3442, 3251, 1639, 1573, 1462, 1280, 992 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 11.41 (1H, s, 2-O*H*), 10.03 (1H, s, C*H*O), 7.18 (1H, d, J = 9.0 Hz, H-4), 6.88 (1H, d, J = 9.0 Hz, H-3), 6.72 (1H, t, J = 11.2 Hz, H-8), 6.45 (1H, dd, J = 14.9, 10.7 Hz, H-10), 6.33 (1H, d, J = 11.0 Hz, H-7), 6.03 (1H, dd, J = 15.1, 10.7 Hz, H-11), 5.97 (1H, dd, J = 14.9, 11.5 Hz, H-9), 5.86 (1H, dt, J = 15.1, 7.0 Hz, H-12), 4.98 (1H, br s, 5-O*H*), 2.09 (2H, br q, J = 7.1 Hz, H-13), 1.38 (2H, m, H-14), 1.28 (2H, m, H-15), 1.26 (2H, m, H-16), 0.88 (3H. t, J = 7.0 Hz, H-17); ^{13}C NMR (125 MHz, $CDCl_3$) δ 196.3 (d, *C*HO), 157.1 (s, C-2), 145.1 (s, C-5), 139.6 (d, C-12), 139.2 (d, C-10), 137.8 (d, C-8), 129.9 (d, C-11), 125.7 (d, C-4), 124.7 (d, C-9), 124.6 (s, C-6), 118.2 (d, C-3), 117.8 (d, C-7), 117.5 (s, C-1), 32.9 (t, C-13), 31.4, t, C-15), 28.7 (t, C-14), 22.5 (t, C-16), 14.0 (q, C-17); HRMS (ESITOF) m/z 285.1498 $[M - H]^-$ (calcd for $C_{18}H_{21}O_3$, 285.1496).

5-Deoxyaurocitrin (3): pale brown solid; UV–vis (MeOH) λ_{max} (log ε) 227 (4.28), 269 sh (4.47), 285 (4.48), 307 sh (4.46), 380 (4.17) nm; IR (KBr) ν_{max} 3452, 1644, 1453, 1236, 997 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 11.94 (1H, s, 2-O*H*), 10.33 (1H, s, C*H*O), 7.44 (1H, t, J = 8.0 Hz, H-4), 7.00 (1H, d, J = 7.7 Hz, H-5), 7.00 (1H, d, J = 15.2 Hz, H-7), 6.84 (1H, d, J = 8.3 Hz, H-3), 6.71 (1H, dd, J = 15.2, 10.4 Hz, H-8), 6.40 (1H, dd, J = 14.9, 10.4 Hz, H-10), 6.32 (1H, dd, J = 14.9, 10.4 Hz, H-9), 6.15 (1H, ddt, J = 15.1, 10.4, 1.3 Hz, H-11), 5.85 (1H, dt, J = 15.1, 7.1 Hz, H-12), 2.14 (2H, br q, J = 7.1 Hz, H-13), 1.42 (2H, m, H-14), 1.30 (2H, m, H-15), 1.29 (2H, m, H-16), 0.89 (3H, q, J = 7.0 Hz, H-17); ^{13}C NMR (125 MHz, $CDCl_3$) δ 195.2 (d, *C*HO), 163.0 (s, C-2), 142.9 (s, C-6), 138.2 (d, C-12), 137.1 (d, C-4), 136.5 (d, C-10), 136.3 (d, C-8), 130.1 (d, C-11), 129.8 (d, C-9), 124.7 (d, C-7), 118.2 (d, C-5), 117.1 (s, C-1), 116.6 (d, C-3), 33.0 (t, C-13), 32.1 (t, C-15), 28.9 (t, C-14), 22.5 (t, C-16), 14.1 (q, C-17); HRMS (ESITOF) m/z 269.1545 $[M - H]^-$ (calcd for $C_{18}H_{21}O_2$, 269.1547).

Compound 4: pale brown solid; UV–vis (MeOH) λ_{max} (log ε) 213 (4.15), 272 (4.39), 324 sh (4.13), 392 (4.14) nm; IR (KBr) ν_{max} 3443, 1726 (w), 1651, 1621, 1304, 1247, 1198, 987 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 11.37 (1H, s, 2-O*H*), 10.27 (1H, s, C*H*O), 7.54 (1H, dd, J = 8.9, 0.7 Hz, H-4), 7.01 (1H, dd, J = 15.5, 10.8 Hz, H-10), 6.84 (1H, br s, H-7), 6.81 (1H, d, J = 8.9 Hz, H-3), 6.35 (1H, d, J = 15.5 Hz, H-9), 6.22 (1H, dd, J = 15.0, 10.8 Hz, H-11), 6.01 (1H, dt, J = 15.0, 7.1 Hz, H-12), 2.19 (2H, br q, J = 7.2 Hz, H-13), 1.45 (2H, m, H-14), 1.33 (2H, m, H-15), 1.31 (2H, m, H-16), 0.90 (3H, q, J = 6.9 Hz, H-17); ^{13}C NMR (125 MHz, $CDCl_3$) δ 192.8 (d, *C*HO), 159.6 (s, C-2), 158.9 (s, C-8), 148.8 (s, C-5), 140.1 (d, C-12), 133.6 (d, C-10), 131.1 (s, C-6), 129.7 (d, C-11), 119.7 (d, C-4), 116.8 (d, C-9), 113.5 (d, C-3), 111.2 (s, C-1), 100.0 (d, C-7), 33.0 (t, C-13), 31.5 (t, C-15), 28.8 (t, C-14), 22.5 (t, C-16), 14.0 (q, C-17); HRMS (ESITOF) m/z 283.1335 $[M - H]^-$ (calcd for $C_{18}H_{19}O_3$, 283.1334).

Compound 5: yellow solid; IR (KBr) ν_{max} 3453, 1712, 1693, 1650, 1619, 1292, 1247, 1198, 992, 816, 732 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 11.37 (1H, s, 2-O*H*), 10.26 (1H, s, C*H*O), 7.54 (1H, dd, J = 8.9, 0.9 Hz, H-4), 6.99 (1H, dd, J = 15.5, 10.9 Hz, H-10), 6.84 (1H, br s, H-7), 6.82 (1H, d, J = 8.9 Hz, H-3), 6.36 (1H, d, J = 15.5 Hz, H-9), 6.24 (1H, dd, J = 14.9, 10.9 Hz, H-11), 5.98 (1H, dt, J = 14.9, 7.1 Hz, H-12), 2.39 (2H, t, J = 7.4 Hz, H-16), 2.22 (2H, br q, J = 7.1 Hz, H-13), 1.69 (2H, m, H-15), 1.52 (2H, m, H-14); ^{13}C NMR (125 MHz, $CDCl_3$) δ 192.8 (d, *C*HO), 177.0 (s, C-17), 159.6 (s, C-2), 158.8 (s, C-8), 148.8 (s, C-5), 138.8 (d, C-12), 133.3 (d, C-10), 131.1 (s, C-6), 130.2 (d, C-11), 119.7 (d, C-4), 117.2 (d, C-9), 113.6 (d, C-3), 111.2 (s, C-1), 100.3 (d, C-7), 33.4 (t, C-16), 32.6 (t, C-13), 28.5 (t, C-14), 24.3 (t, C-15); HRMS (ESITOF) m/z 337.1052 $[M + Na]^+$ (calcd for $C_{18}H_{18}O_5Na$, 337.1046).

Compound 6: pale brown solid; UV (MeOH/H_2O, 70:30) λ_{max} 227, 347 nm; ^1H NMR (500 MHz, $CDCl_3$) δ 10.61 (1H, s, 2-O*H*), 7.07 (1H, d, J = 9.0 Hz, H-4), 6.92 (1H, d, J = 9.0 Hz, H-3), 6.56 (1H, br s, 5-O*H*), 6.44 (1H, dd, J = 15.4, 10.5 Hz, H-10), 6.07 (1H, dd, J = 15.3, 10.5 Hz, H-11), 5.82 (1H, dt, J = 15.3, 6.9 Hz, H-12), 5.78 (1H, dd, J = 15.4, 7.6 Hz, H-9), 5.08 (1H, dd, J = 7.6, 3.6 Hz, H-8), 4.95 (1H, d, J = 3.6 Hz, H-7), 3.48 (3H, s, 7-OCH_3), 2.09 (2H, br q, J = 7.2 Hz, H-13), 1.39 (2H, m, H-14), 1.30 (2H, m, H-15), 1.28 (2H, m, H-16), 0.89 (3H, t, J = 7.0 Hz, H-17); ^{13}C NMR (125 MHz, $CDCl_3$) δ 168.4 (s, −*C*OO−), 156.2 (s, C-2), 146.4 (s, C-5), 138.6 (d, C-12), 137.0 (d, C-10), 128.8 (d, C-11), 125.7 (d, C-4), 122.1 (d, C-9), 120.2 (s, C-6), 119.0 (d, C-3), 106.8 (s, C-1), 80.2 (d, C-8), 72.9 (d, C-7), 57.1 (q, 7-O*C*H_3), 32.6 (t, C-13), 31.4 (t, C-15), 28.7 (t, C-14), 22.5 (t, C-16), 14.0 (q, C-17); HRMS (ESITOF) m/z 335.1526 $[M + Na]^+$ (calcd for $C_{19}H_{24}O_5Na$, 355.1516).

Biological Assays. Assay for activity against *Plasmodium falciparum* (K1, multidrug-resistant strain) was performed using the microculture radioisotope technique described by Desjardins.[12] Growth inhibitory activity against *Mycobacterium tuberculosis* $H_{37}Ra$ was performed using the microplate Alamar Blue assay (MABA) described by Collins and Franzblau.[13] Cytotoxicity against KB cells (oral human epidermoid carcinoma), BC cells (human breast cancer), NCI-H187 cells (human small cell lung cancer), and Vero cells (African green monkey kidney fibroblasts) was evaluated using the colorimetric method.[14]

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Supporting Information Available: NMR spectra of compounds **1**–**6**. This material is available free of charge via the Internet at http://pubs.acs.org.

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