Dicerandrols, New Antibiotic and Cytotoxic Dimers Produced by the Fungus *Phomopsis longicolla* Isolated from an Endangered Mint

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Bioassay-guided fractionation of the organic extract from a culture of *Phomopsis longicolla*, an endophytic fungus of the endangered mint *Dicerandra frutescens*, led to the isolation of dicerandrols A, B, and C. Extensive NMR and HRFABMS experiments were used to identify these new yellow antibiotic and cytotoxic compounds as 2,2′-dimeric tetrahydroxanthones.

Dicerandra frutescens (Labiatae), a rare mint plant on the Federal Endangered Species List, is found at about a dozen sites within an area of only a few hundred acres in central Florida. These sites may represent the islands upon which *D. frutescens* spent its evolutionary history that formed the central Florida highlands prior to the lowering of the world's ocean levels.[1,2] Earlier studies of *D. frutescens* showed the odiferous plant to be free of injury from insects. Twelve closely related monoterpenes, including the previously undescribed compound (+)-*trans*-pulegol, have been characterized from *D. frutescens* leaf extract and shown to act as chemical defenses against insects.[1,2] Because of its Federal Endangered Species status and the rich chemical history of *D. frutescens*, we chose to investigate the endophytic fungi of *D. frutescens* and five other mint species known to grow in the same region as *D. frutescens* for the production of bioactive compounds.

Endophytic fungi, fungi that grow in the intercellular spaces of higher plants, are recognized as one of the most chemically promising groups of fungi in terms of diversity and pharmaceutical potential.[3,4] A very recent analysis of the magnitude of fungal species suggests that fungal endophytes alone are a hyperdiverse group of fungi with an estimated range of 30−150 species per host.[5] Furthermore, it has been noted that a subset of the endophytic fungi may be host−species specific. Therefore, the entire plant community likely harbors a major portion of fungal diversity, and the extinction of even one plant species could result in the loss of several host-specific fungal endophytes.

One fungus, MMW29, isolated from the stem of a *D. frutescens* plant, exhibited activity against *Staphylococcus aureus* and *Bacillus subtilis* when grown in shake culture in potato dextrose broth (PDB). The fungus was identified as *Phomopsis longicolla* by isolation of DNA from the fungal mycelium, PCR amplification of the internal transcribed spacer regions ITS1 and ITS2, and comparison of the resulting ITS1 and ITS2 sequence with deposited sequences using a BLAST[6] search. Using bioassay-guided fractionation, three closely related yellow antibiotics trivially named dicerandrols were isolated.

Results and Discussion

Compound **1** is an amorphous yellow powder with the molecular formula $C_{34}H_{34}O_{14}$ (HRMSFAB (*m*/*z*) $[M + H]^+$ calcd for $C_{34}H_{35}O_{14}$, 667.2027; found, 667.2015), and since the ^{13}C NMR shows only 17 carbon resonances, it must be a symmetrical dimer. The UV spectrum (λ_{max} 264 and 340 nm) closely resembles the UV spectrum of the secalonic acids, suggesting compound **1** may be structurally related to the secalonic acids.[7] Analysis of the ^1H and ^{13}C NMR data for **1** indicated the presence of two carbonyls (an acetate at δ_C 171.4 and a ketone at δ_C 189.1), a tetrasubstituted aromatic ring, an enol, two methyl groups (one of which is part of the acetate), two methylene groups, two methines, and one quaternary carbon in each monomer. The presence of an enolized β-diketone is further suggested by a red-brown ferric chloride test and the IR absorption at 1608 cm^{-1}. On the basis of the molecular formula, the dimer **1** contains 18 degrees of unsaturation and, therefore, 9 degrees of unsaturation per monomer. The aromatic ring, one carbonyl, and the β-diketone system account for seven of the nine unsaturations of the monomer unit. Therefore, the monomer must contain two additional rings. In addition, the ^1H NMR showed two H-bonded hydroxyl groups (δ_H 13.94 and 11.83) and one free hydroxy (δ_H 3.36) in each monomer. From a combination of $^1H-^1H$ RelayH, HMQC, and HMBC experiments, the relative stereostructure of compound **1** was deduced as discussed below.

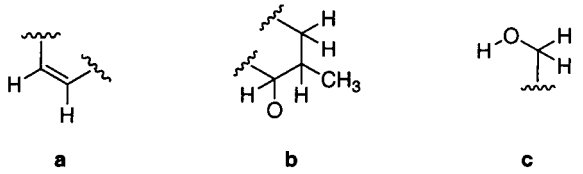

Figure 1. Spin systems elucidated by $^1H-^1H$ RelayH and HMQC experiments for dicerandrol A (**1**).

The ^1H NMR spectrum of **1** in conjunction with $^1H-^1H$ RelayH (COSY-45) and pulsed-field gradient HMQC (PFG-HMQC) identified three spin systems: from C-3 to C-4 (**a**); from C-5 to C-7 including C-11 (**b**); and from C-12 to the hydroxy on C-12 (**c**), as shown in Figure 1.

The connectivity of the spin systems into the carbon skeleton of compound **1** was established using HMBC correlations. The acetate group, composed of the carbonyl carbon at δ_C 171.4 and the methyl carbon at δ_C 21.0 (protons at δ_H 2.03), is attached to the oxygenated methine carbon at δ_C 71.8 in spin system **b** on the basis of the HMBC correlation from the proton at δ_H 5.61 (H-5) to the carbonyl carbon at δ_C 171.4. The protons at δ_H 2.46 (H-6) and δ_H 2.33 (H-7α) are correlated to the quaternary carbon at δ_C 179.9 (C-8), which is part of the enolized β-diketone system. In addition, the protons at δ_H 2.33 and 2.50 (H-7α and H-7β, respectively) correlate to the carbon at δ_C 102.3 (C-8a). Therefore, the carbon at δ_C 102.3 is linked to the

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carbon at δ_C 179.9, which is adjacent to the carbon at δ_C 34.2 (C-7) of spin system **b**. The proton at δ_C 5.61 (H-5) also correlates to the carbon at δ_C 102.3, suggesting the presence of either a five- or six-membered ring. To account for the correlation of the proton signal at δ_H 3.90 to both δ_C 71.8 and 102.2, spin system **b** must be part of a six-membered ring completed by a quaternary carbon (δ_C 83.8) between C-8a and C-5. Spin system **c** attaches to this six-membered ring through this quaternary carbon, as indicated by the long-range correlation of δ_H 3.90 to δ_C 83.8. The proton at δ_H 5.61 displays a weak four-bond correlation to the carbonyl carbon at δ_C 189.1, confirming the enolized β-diketone system. This partial structure accounts for 11 of the 17 carbons in the monomer unit, with the remaining six carbons part of the tetrasubstituted aromatic ring.

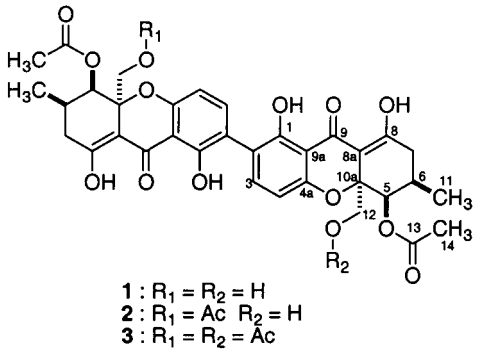
Because there are no remaining carbons and the unsaturation index indicates that compound **1** contains one more ring, the ketone must also be directly connected to the aromatic ring. A long-range HMBC correlation from the aromatic proton signal at δ_H 6.43 (H-4) to the ketone carbon lends further evidence to this structure. The aromatic ring contains two downfield carbons at δ_C 160.3 and 158.9, suggestive of oxygenated carbons: one carbon is attached to a hydroxyl group, while the other carbon attaches through an ether linkage to the carbon at δ_C 83.8. The UV spectrum, which is consistent with a 5-hydroxychromanone nucleus,[8] locates the phenolic hydroxyl on C-1 and the other oxygenated carbon as C-4a. The phenolic hydroxyl group is hydrogen-bonded to the ketone based on its downfield chemical shift of δ_H 11.83. The HMBC correlations of the phenolic hydroxyl group to δ_C 160.3, 118.5, and 107.3 indicate the hydroxyl group is attached to the oxygenated carbon at δ_C 160.3 (C-1). Therefore, the other oxygenated aromatic carbon signal at δ_C 158.9 corresponds to C-4a and attaches through an ether linkage to C-10a. Both aromatic protons of spin system **a** show long-range correlations to δ_C 158.9 and 118.5, indicating that spin system **a** is flanked by these two carbons and that δ_C 118.5 corresponds to C-2. Therefore, C-9a, which is attached to the ketone carbonyl, resonates at δ_C 107.3. Only the proton signal at δ_H 6.43 of spin system **a** correlates to δ_C 107.3, positioning this proton *para* to the phenolic OH and the proton for δ_H 7.39 *meta* to the phenolic OH. The final substitution on the aromatic ring is the point of attachment for dimerization, and the only position available is C-2. Therefore compound **1**, trivially named dicerandrol A, must be the 2,2′ symmetrical dimer with the relative stereochemistry shown.

Compound **3**, molecular formula $C_{38}H_{38}O_{16}$ (HRMSFAB (*m*/*z*) $[M + H]^+$ calcd for $C_{38}H_{39}O_{16}$, 751.2238; found, 751.2235), contains four more carbons, four more protons, and two more oxygens than compound **1**, resulting in a mass difference of 84. However, only 19 carbon signals are present in the ^{13}C NMR of compound **3**, indicating that like compound **1** it is a symmetrical dimer. The IR, UV, and red-brown $FeCl_3$ reaction suggest compound **3** is similar to compound **1**. Both the ^1H NMR and ^{13}C NMR spectra of compound **3** closely resemble those of compound **1** with a few exceptions. In the ^{13}C NMR spectrum of compound **3** two additional signals at δ_C 171.1 and 20.9 are present, suggesting the addition of an acetate group to the monomer unit of compound **1**. Furthermore, C-10a shifts upfield from δ_C 83.8 to 82.0, typical of a β-shift observed upon acetylation.[9] The ^1H NMR spectrum of compound **3** supports the presence of an acetate by the addition of a methyl singlet at δ_H 2.00, the downfield shift of the C-12 methylene proton signals from δ_H 3.55 and 3.90 to δ_H 4.20 and 4.99, and the absence of the CH_2-O*H* proton signal at δ_H 3.36. Therefore, compound **3**, trivially named dicerandrol C, is the 12,12′-diacetate derivative of compound **1**.

Table 1. ^{13}C NMR Data in CD_3CN (δ in ppm)

	1[a]	**2**[b]		**3**[a]
carbon no.	symmetric dimer	unsymmetric dimer		symmetric dimer
1,1′	160.3	160.3	160.3	160.3
2,2′	118.5[c]	118.5[c]	118.9	118.8
3,3′	141.1	141.0	141.0	141.2
4,4′	109.0	108.9	108.7	108.8
4a,4a′	158.9	158.9	158.5	158.6
5,5′	71.8	71.8	71.6	71.6
6,6′	28.5	28.5	28.5	28.5
7,7′	34.1	34.1	34.1	34.1
8,8′	179.9	180.1	180.0	180.4
8a,8a′	102.3	102.3	101.4	101.5
9,9′	189.1	189.0	189.0	189.0
9a,9a′	107.3	107.3	107.3	107.3
10,10′				
10a,10a′	83.8	83.8[d]	82.0[d]	82.0
11,11′	17.8	17.8	17.7	17.7
12,12′	65.8	65.8	66.0	66.1
13,13′	171.4	171.4	171.3[e]	171.3[g]
14,14′	21.0	21.0	21.0[f]	21.0[h]
15,15′			171.1[f]	171.1[g]
16,16′			20.9[e]	20.9[h]

[a] Chemical shifts are equivalent for both monomer units. [b] Chemical shifts differ between monomer units and are listed in separate columns. [c] Signals buried under solvent signal; assignment based on HMBC experiment. [d–h] Signals are interchangeable.



The IR and UV spectra along with the $FeCl_3$ test for compound **2** indicate that it is closely related to both compounds **1** and **3**. HRFABMS data (HRMSFAB (*m*/*z*) $[M + H]^+$ calcd for $C_{36}H_{37}O_{15}$, 709.2132; found, 709.2133) for compound **2** indicate a molecular formula of $C_{36}H_{36}O_{15}$, corresponding to a mass difference of 42 from compound **1**. This difference is suggestive of the addition of a single acetate group to compound **1**. As clearly shown in Table 1, the ^{13}C NMR spectrum of compound **2** appears to be a combination of the ^{13}C NMR spectra of compounds **1** and **3**. The ^1H NMR spectrum of compound **2** also has the appearance of the ^1H NMR spectrum of compound **1** superimposed on the spectrum of compound **3**. In addition, the optical rotation ($[\alpha]^{25}_D$ −6.5°) of compound **5** is the sum of the optical rotations of compounds **1** and **3** ($[\alpha]^{25}_D$ −50.9° and $[\alpha]^{25}_D$ +44.3°, respectively).[10] The mass difference of 42, the loss of symmetry in both the ^1H and ^{13}C NMR spectra, and the composite appearance of the NMR spectra indicate compound **2**, dicerandrol B, is an unsymmetrical dimer consisting of one monomer unit from compound **1** and one unit from compound **3**, as illustrated.

Because of the simplicity of the ^1H NMR spectrum and the minimal overlap of proton signals, the relative stereochemistry of dicerandrol C (**3**) was determined using ^1H−

Table 2. Antimicrobial Activity and Cytotoxicity Data for Dicerandrols A, B, and C

	zones of inhibition[a] (mm)		IC_{100} (μg/mL)	
	B. subtilis	*S. aureus*	A549	HCT116
1	11.0	10.8	7.0	7.0
2	9.5	8.5	1.8	1.8
3	8.0	7.0	1.8	7.0
nystatin[b]	12.0			
neomycin[c]		9.0		
etoposide			30.0	125.0

[a] Zones of inhibition resulting from 300 μg/disk. [b] Nystatin control disk contains 100 units (approximately 30 μg). [c] Neomycin control disk contains 30 μg.

^{1}H coupling constants and a NOESY experiment. Similar NMR shifts and coupling constants were observed for compounds **1** and **2**, and thus they are assumed to have the same relative stereochemistry. The coupling constants $J_{6,7\alpha} = 11.0$ Hz and $J_{6,7\beta} = 6.5$ Hz are consistent with a *trans*-diaxial relationship between H-6 and H-7β; thereby the CH_3-11 must be in the equatorial position. The signal for H-6 is also split by a 1.5 Hz coupling constant to H-5, establishing H-5 as equatorial and the acetoxy as axial. The hydroxymethyl group (C-12) is in a *cis*-position with respect to proton H-6, as shown by the presence of a NOESY correlation from H-12 to H-6. Therefore the hydroxymethyl moiety is in the axial position. This stereochemistry is analogous to the structures of all known secalonic acids and ergochromes in which the C-6 methyl group is *trans* to the C-10a carbomethoxy group.[11] Secalonic acids B and E are the most closely related compounds and possess the same relative configuration of substituents as compound **3**.

Dicerandrols A, B, and C (**1**, **2**, and **3**) are structurally related to the ergochromes and secalonic acids in that they contain the same tricyclic C_{15} system with similar arrangement of substituents. While one of the final steps in the formation of the secalonic acids is methylation of the carboxylic acid to give the C-10a carbomethoxy functionality,[12] compound **1** could result from a reduction of the C-10a carboxylic acid group to the hydroxymethyl moiety. The dicerandrols (**1**−**3**) are the first reported compounds with this tricyclic C_{15} skeleton with a reduced C-12 functionality.

Dicerandrols A, B, and C (**1**−**3**) exhibit antibacterial activity against both *Staphylococcus aureus* and *Bacillus subtilis* but are inactive against the fungus *Geotrichum candidum* and the yeast *Saccharomyces cerevisiae* at 300 μg/disk (Table 2). The compounds also possess modest activity in two human cancer cell lines, A549 and HCT-116. Both secalonic acids B and E, the two most closely related compounds, have been reported to show weak cytostatic activity.[13] As can be seen in Table 2, the antibacterial activity of these compounds decreases upon successive acetylation; the C-12 monoacetate (**2**) exhibits less activity than **1**, and the C-12,C-12′-diacetate (**3**) is the least active of all three related compounds.

As noted in the Introduction, the continued loss of vascular plant habitat around the world is undoubtedly causing the extinction of fungi before they can be studied.[14] The isolation of these new bioactive compounds from an endophytic fungus of the endangered *D. frutescens* underscores the need for conservation of all organisms.

Experimental Section

General Experimental Procedures. ^{1}H and 2D NMR experiments were performed on a Varian Unity 500 MHz spectrometer, while ^{13}C NMR experiments (100 MHz) were performed on a Varian Unity 400 MHz spectrometer. NMR spectra were recorded using CD_3CN solutions, and the chemical shifts were referenced relative to the corresponding solvent signals (δ_H 1.94 for ^{1}H NMR and δ_C 1.39 for ^{13}C NMR). The IR spectra were recorded on a Perkin-Elmer 16PC FTIR spectrometer. The optical rotations were measured on a Perkin-Elmer 241 polarimeter. The UV spectra were recorded on a Spectronic Genesys 2 spectrophotometer. Mass spectral data were acquired by the University of Illinois, Urbana, Mass Spectrometry Facility. DNA sequencing was performed by the BioResources Center at Cornell University.

Fungal Material. The unidentified endophytic fungus MMW29 was isolated from the mint *Dicerandra frutescens* in September 1995. A stem segment from *D. frutescens* was surface sterilized by successive submersion in 70% EtOH for 1 min, 10% bleach for 3 min, and then twice in sterile water.[15] After sterilization, the stem was placed on a potato dextrose agar (PDA) plate, and successive subculturing of the outgrowing fungi resulted in a pure culture initially coded MMW29. A voucher of the fungus has been deposited in the Cornell University fungal herbarium, CUP (ppathw3.cals.cornell.edu/CUPpages/CUP.html).

Identification of MMW29 was accomplished by sequencing of ITS1 and ITS2 (internal transcribed regions) following the general procedures outlined by Lee and Taylor[16] for the isolation of DNA and by White et al.[17] for the amplification and sequencing of rRNA genes. After a 2-week fermentation of MMW29 in potato dextrose broth, the mycelium was filtered using cheesecloth and 2−4 mL of lysis buffer (2% Triton X-100, 1% SDS, 100 mM NaCl, 10 mM Tris, pH 8.0, 1 mM EDTA), and 1 g of glass beads was added to approximately 0.2−0.6 g of mycelium in a 50 mL conical tube. The sample was vortexed for 2−7 min and then centrifuged for 10 min. Then 0.5 mL of supernatant was transferred into a microfuge tube, 0.5 mL of phenol/chloroform was added, and the tube was vortexed for 1 min. The aqueous phase was transferred to a clean microfuge tube and washed with $CHCl_3$. The aqueous phase was then combined with 2 volumes of absolute EtOH and centrifuged for 15 min at 4 °C. The supernatant was removed, and the pellet was washed with 70% EtOH. The pellet was then resuspended in 30 μL of T.E. buffer (10 mM Tris, pH 8.0, 1 mM EDTA).

The DNA was amplified by PCR using primers ITS1 and ITS4 (ITS1: TCCGTAGGTGAACCTGCGG and ITS4: TCCTCCGCTTATTGATATGC). Each 100 μL amplification reaction mixture contained 2 μL of 1:10 dilution of DNA, 10 μL of 10× reaction buffer (100 mM Tris-HCl, pH 8.85, 250 mM KCl, 50 mM $(NH_4)_2SO_4$, 20 mM $MgSO_4$), 2 μL of ITS1 (50 μM), 2 μL of ITS4 (50 μM), 2 μL of dNTP (25 mM); 0.5 μL of Pwo DNA polymerase ((1−5) × 10^3 units/mL), and 81.6 μL of H_2O. All reaction mixtures were incubated in a thermal cycler for 5 min at 94 °C and then subjected to 35 cycles of 1.5 min at 92 °C, 1.5 min at 50 °C, and 2 min at 72 °C, and then a final extension of 10 min at 72 °C. PCR products were gel purified using QIAquick gel extraction kit (QIAGEN).

DNA sequencing of each product was done using ITS1, ITS2, ITS3, and ITS4 as primers. The resulting consensus sequence is as follows:

5′-AACCTGCGGAGGGATCATTGCTGGAACGCGCTT-CGGCGCACCCAGAAACCCTTTGTGAACTTATACCTA-TTTGTTGCCTCGGCGTAGGCCGGCCTTTTGTGA-CAAAGGCCCCCTGGAAACAGGGAGCAGCCCGCC-GGCGGCCAACTAAACTCTTGTTTCTATAGTGAATCTCT-GAGTAAAAACATAAATGAATCAAAACTTTCAACAA-CGGATCTCTTGGTTCTGGCATCGATGAAGAACGCA-GCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGT-GAATCATCGAATCTTTGAACGCACATTGCGCCCTCT-GGTATTCCGGAGGGCATGCCTGTTCGAGCGTCATTT-CAACCCTCAAGCCTGGCTTGGTGATGGGGCACTGCC-TTCTAACGAGGGCAGGCCCTGAAATCTAGTGGCGAG-CTCGCTAGGACCCCGAGCGTAGTAGTTATATCTCGTT-CTGGAAGGCCCTGGCGGTGCCCTGCCGTTAAACCCCC-AACTTCTGAAAATTTGACCTCGGATCAGGTAGG-AATACCCGCTGAACTTAAGCATATCAATA-3′

Comparison of the sequence with deposited sequences using BLAST resulted in 97% identity with the ITS regions of *Phomopsis longicolla*.

A subculture of MMW29 was used to inoculate six 500 mL Erlenmeyer flasks each containing 250 mL of potato dextrose broth (Difco). The cultures were grown at room temperature under shake conditions for 15 days.

Extraction and Isolation. The fungal cultures were filtered using cheesecloth, and the filtrate was adjusted to pH 7. The filtrate was extracted three times with EtOAc in a 1:1 ratio. The EtOAc layer was washed with saturated NaCl solution, dried over $MgSO_4$, and concentrated in vacuo to yield 480 mg of crude extract. Using a modified Kupchan scheme[18] (hexanes, CCl_4, CH_2Cl_2, MeOH−H_2O), the crude extract was subjected to liquid−liquid partitioning. The CCl_4 layer (222.4 mg) was chromatographed on a C-18 column (6.0 × 1.7 cm) using a step gradient of CH_3CN−H_2O (40 mL; 50% to 100% CH_3CN in 10% increments) collecting 10 mL fractions. Biologically active fractions were combined based on TLC. Fractions 4 and 5 were combined and subjected to reversed-phase HPLC (C-18; 25 cm × 10 mm; 5 μm) using 75:25 CH_3CN−H_2O to yield 27.9 mg of compound **1** (t_R 8.8 min). Fractions 6−9 (140.2 mg) were combined and subjected to HPLC to yield an additional 14.6 mg of compound **1** and 69.9 mg of compound **2** (t_R 11.8 min). Fractions 10−13 were combined and subjected to reversed-phase HPLC using 80:20 CH_3CN−H_2O, yielding 5.8 mg of compound **3** (t_R 7.9 min).

Dicerandrol A (**1**): $[\alpha]^{25}_D$ −50.9° (*c* 0.01, $CHCl_3$); UV (MeOH) λ_{max} 265, 343 nm; IR ($CHCl_3$) ν_{max} 1744, 1608, 1588, 1562, 1434, 1372, 1286, 1236, 1050 cm^{-1}; ^1H NMR (CD_3CN, 500 MHz) δ 13.94 (2H, bs, enolic OH), 11.83 (2H, s, phenolic OH), 7.39 (2H, d, J = 8.5 Hz, H-3, H-3′), 6.43 (2H, d, J = 8.5 Hz, H-4, H-4′), 5.61 (2H, d, J = 1.5 Hz, H-5, H-5′), 3.90 (2H, dd, J = 4.5, 13.0 Hz, H-12α, H-12′α), 3.55 (2H, dd, J = 7.0, 13.0 Hz, H-12β, H-12′β), 3.36 (2H, m, CH_2-O*H*), 2.5 (2H, m, H-7β, H-7′β), 2.46 (2H, m, H-6, H-6′), 2.33 (2H, m, H-7α, H-7′α), 2.03 (6H, s, CH_3-14, CH_3-14′), 1.00 (6H, d, J = 6.5 Hz, CH_3-11, CH_3-11′); ^{13}C NMR (Table 1); HRMSFAB (*m*/*z*) $[M + H]^+$ calcd for $C_{34}H_{35}O_{14}$, 667.2027; found, 667.2015.

Dicerandrol B (**2**): $[\alpha]^{25}_D$ −6.5° (*c* 0.01, $CHCl_3$); UV (MeOH) λ_{max} 265, 341 nm; IR ($CHCl_3$) ν_{max} 1744, 1610, 1588, 1562, 1434, 1372, 1324, 1286, 1236, 1232, 1048 cm^{-1}; ^1H NMR (CD_3CN, 500 MHz) δ 13.97 (2H, bs, enolic OH), 11.82 (1 H, s, phenolic OH C-2), 11.76 (1H, s, phenolic OH C-2′), 7.39 (2H, d, J = 8.5 Hz, H-3, H-3′), 6.43 (1H, d, J = 8.5 Hz, H-4), 6.37 (1H, d, J = 8.5 Hz, H-4′), 5.61 (1H, d, J = 1.5 Hz, H-5), 5.54 (1H, d, J = 1.5 Hz, H-5′), 4.49 (1H, d, J = 13.0 Hz, H-12′α), 4.19 (1H, d, J = 13.0 Hz, H-12′β), 3.90 (1H, dd, J = 5.5, 13.0 Hz, H-12α), 3.55 (1H, dd, J = 7.5, 13.0 Hz, H-12β), 3.36 (1H, t, J = 6.5 Hz, CH_2-O*H*), 2.47 (4H, m, H-7α, H-7β, H-7′α, H-7′β), 2.35 (2H, m, H-6, H-6′), 2.03 (3H, s, CH_3-14′), 2.03 (3H, s, CH_3-16′), 2.00 (3H, s, CH_3-14), 1.01 (3H, d, J = 6.0 Hz, CH_3-11), 1.00 (3H, d, J = 6.0 Hz, CH_3-11′); ^{13}C NMR (Table 1); HRMSFAB (*m*/*z*) $[M + H]^+$ calcd for $C_{36}H_{37}O_{15}$, 709.2132; found, 709.2133.

Dicerandrol C (**3**): $[\alpha]^{25}_D$ +44.3° (*c* 0.003, $CHCl_3$); UV (MeOH) λ_{max} 263, 340 nm; IR ($CHCl_3$) ν_{max} 1746, 1610, 1588, 1562, 1436, 1370, 1324, 1286, 1236, 1232, 1092, 1046 cm^{-1}; ^1H NMR δ 13.95 (2H, bs, enolic OH), 11.78 (2H, s, phenolic OH), 7.39 (2H, d, J = 8.0 Hz, H-3, H-3′), 6.37 (2H, d, J = 8.0 Hz, H-4, H-4′), 5.54 (2H, d, J = 1.5 Hz, H-5, H-5′), 4.49 (2H, d, J = 13.0 Hz, H-12α, H-12′α), 4.20 (2H, d, J = 13.0 Hz, H-12β, H-12′β), 2.53 (2H, dd, J = 6.5, 18.0 Hz, H-7α, H-7′α), 2.45 (2H, m, H-6, H-6′), 2.35 (2H, dd, J = 11.0, 18.0 Hz, H-7β, H-7′β), 2.03 (6H, s, CH_3-14, CH_3-14′ or CH_3-16, CH_3-16′), 2.00 (6H, s, CH_3-14, CH_3-14′ or CH_3-16, CH_3-16′), 1.01 (6H, d, J = 7.0 Hz, CH_3-11, CH_3-11′); ^{13}C NMR (Table 1); HRMSFAB (*m*/*z*) $[M + H]^+$ calcd for $C_{38}H_{39}O_{16}$, 751.2238; found, 751.2235.

Antimicrobial Assay System. The antimicrobial tests were performed using *B. subtilis* and *S. aureus* by the agar diffusion method. Nystatin disks (Difco) containing 100 units (approximately 30 μg) and neomycin disks (Difco) containing 30 μg were used as controls. Disks (6 mm diameter) containing 300 μg of compound were placed on the surface of agar plates seeded with overnight cultures of the test microorganisms. The plates were incubated overnight at 37 °C. After incubation the zones of inhibition were measured and recorded as the diameter of the zone.

Bioassay for Cytotoxicity. HCT-116 (human colon tumor cells) and A549 (human lung tumor cells) were used to determine cytotoxicity. Solutions of pure compounds were made at a concentration of 2.0 mg/mL in methanol. Cell suspensions in RPMI media (Gibco, Life Technologies) supplemented with fetal calf serum, L-glutamine, and gentamycin were diluted to 2.5 × 10^4 cells/mL and added by pipet (150 μL) into 96-well microtiter plates. Cells were then incubated for approximately 24 h in 5% CO_2 at 37 °C. Aliquots of test solutions (50 μL) were added to the microtiter plates and then diluted 4-fold up the plate for a total of eight dilutions. After an additional 72 h incubation period, the cells were fixed with a solution of 10X Hanks' Balanced Salt Solution−37% (w/w) formaldehyde solution−water (1:1:8) for 10 min. Next the cells were stained with 0.0075% crystal violet solution for 15 min, and the concentration resulting in total cell kill (IC_{100}) was read.

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Supporting Information Available: ^1H and ^{13}C NMR spectra for **1**, **2**, and **3**. This material is available free of charge via the Internet at http://pubs.acs.org.

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